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B46

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 15/00, C07H 15/12

A1

(11) International Publication Number: WO 95/28427

(43) International Publication Date: 26 October 1995 (26.10.95)

(21) International Application Number: PCT/US94/04208

(22) International Filing Date: 15 April 1994 (15.04.94)

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(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: CHIMERIC INTERLEUKIN-3/MUTEIN INTERLEUKIN-6 LYMPHOKINE

(57) Abstract

This invention provides a chimeric protein comprising an amino portion having the amino acid sequence of interleukin-3 and a carboxy portion having the amino acid sequence of mutein interleukin-6.

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Chimeric Interleukin-3/Mutein Interleukin-6 Lymphokine

The present invention relates to a chimeric protein comprised of Interleukin-3 and a mutein of Interleukin-6. The mutein of Interleukin-6 (mIL-6) has the first two cysteine residues replaced with any other amino acid residue. The chimera may be constructed according to the following formula:

IL-3--L--mIL-6

in which IL-3 represents Interleukin-3, mIL-6 represents the mutein of Interleukin-6 and L represents the first twenty-two amino acid residues of the Interleukin-6 mutein. (See Figure 2) An example of the nucleic and amino acid sequence of the chimeric IL-3/mIL-6 protein of the present invention is shown below in SEQ. ID. NO. 1. The invention also includes nucleic acid sequences encoding such proteins, plasmids and vectors containing such nucleic acid sequences, cells capable of expressing the protein and methods of using the protein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the three nucleic acid fragments used to construct the chimeric IL-3/mIL-6 protein of the present invention. Line A represents nucleic acid sequences encoding human IL-3. Line B represents the restriction fragment obtained from the IL-3 sequence represented by line A by endonuclease digestion with *Nco*I and *Dde*I. The plasmid containing the fragment is designated p570. Lines C and C' show the 3' end of the IL-3

restriction fragment (line B), which lacks the nucleic acid sequence that encodes the eleven amino acids from the carboxy terminal end of native IL-3 (line A). Lines D and D' represent an oligonucleotide pair that contains the nucleic acid sequences for the last eleven amino acids of IL-3 and the first four amino acids of mIL-6, all of which are forfeited during digestion of the nucleic acid sequences encoding IL-3 with *Nco*I and *Dde*I endonucleases and mIL-6 with *EcoR*II and *Hind*III endonucleases. Line E represents the *EcoR*II/*Hind*III restriction fragment encoding mIL-6 which lacks the first four amino acid residues from the amino terminal end of the molecule. Line E' represents the portion of the pKK223-2 IL-6 SSCC plasmid which contains the nucleic acid sequences that encode the mIL-6 protein which lacks the first four amino acid residues from the amino terminal end of the molecule. Line E* represents the sequence from which the *EcoR*II/*Hind*III restriction fragment (line E) is obtained.

Figure 2 illustrates the relative positions of the IL-3, L and mIL-6 portions of one embodiment of the chimera of the present invention.

Figure 3 illustrates the expression vector pSE420. The pSE420 vector contains the $lacI^q$ gene, which allows for regulated expression in *E.coli* HB101. Transcriptional control is via the *trc* promoter and utilizes the highly efficient translation re-initiation characteristic of mini-cistron systems. The incorporation of upstream anti-termination and g10 ribosome binding sequences ensures high level translation of inserts cloned into its polylinker. Digestion of pSE420 with *Nco*I and *Kpn*I allows subsequent mobilization of the IL-3/mIL-6 chimera, by *Nco*I/*Kpn*I digestion of the IL-3/mIL-6-pKK233-2 plasmid, into this protein expression system.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

In this specification, Interleukin-3 (IL-3) and Interleukin-6 (IL-6) refer to human IL-3 and human IL-6, respectively. The terms IL-3 and IL-6 include proteins described in the literature as having the same name as IL-3 or IL-6. For example, IL-3 is also known as multi-colony-stimulating factor (multi-CSF). IL-6 is also known as interferon-ß-2 (IFN-ß-2), B-cell stimulation factor-2 (BSF-2), B-cell hybridoma/plasmacytoma growth factor (HPGF or HGF), 26 kDa protein and hepatocyte stimulating factor (HSF).

The DNA and amino acid sequences of IL-3 are published and may be constructed by methods known in the art; see, for example, PCT publication WO 88/00598, published 28 January 1998 and PCT publication WO92/04455, published 19 March 1992.

The amino acid sequence of IL-6 has been described in the literature; see, for example, Figure 2A of Brakenhoff et al., Journal of Immunology 139, 4116-4121 (1987) and Figure 1 of Clark et al., PCT publication WO 88/00206, published 14 January 1988. These references also contain the cDNA sequence that corresponds to native IL-6 mRNA.

A mutein of IL-6 in which the first two cysteine residues are replaced by other amino acids has been described by Skelly et al., in co-pending U.S. patent application 07/907,710, which is incorporated herein by reference. mIL-6 has also been described in the literature; see for example, Dagan et al., *Protein Expression and Purification* 3, 290-294 (1992) and Snouwaert, J., et al., *J. Immunol.* 146, 585-591 (1991). These references define native IL-6 as a protein having 185 amino acids starting with alanine at amino acid position one.

mIL-6 is a mutein wherein the cysteine residues corresponding to amino acid positions 45 and 51 of native IL-6 have been replaced by other amino acids, while th cysteine residues corresponding to amino acid positions 74 and 84 have been retained. Preferably, the cysteine residues are replaced by neutral amino acids such as serine or alanine.

DNA sequences that encode native IL-3 and IL-6 include, but are not limited to, mammalian sources such as murine, pan and human sequences.

The term "chimera" or "chimeric protein" in this specification is understood to refer to a non-naturally occurring protein that is formed by joining one genetically distinct protein to another genetically distinct protein, end to end, in such a way that the biological activity of both proteins is retained or enhanced.

The term "fusion protein" in this specification is understood to refer to a protein that is produced in a system in which the desired protein is linked to a fusion partner, usually for the purpose of expediting expression or purification. Some suitable fusion partners include *trp*E, b-galactosidase, Protein A, maltose binding protein, etc. Once the fusion protein is produced, the desired protein may be cleaved from the fusion partner.

The words "amino acid" in this specification are understood to mean the approximately 21 naturally occurring a-amino acids or their analogs.

Preparation

The chimeric IL-3/mIL-6 protein and fragments thereof may be prepared by methods known in the art. A preferred method of preparing the chimeric protein of the present invention involves isolating DNA sequences that encode IL-3 and mIL-6, joining the IL-3 and mIL-6 encoding sequences in frame to form a single nucleic acid sequence that encodes the IL-3/mIL-6 chimera; amplifying or cloning the DNA in a suitable host; expressing the DNA in a suitable host; and harvesting the protein.

More specifically, a chimeric IL-3/mIL-6 nucleic acid sequence may be constructed as follows:

1) the major portions of the IL-3 and mIL-6 genes are excised with restriction endonucleases from plasmids containing the genes;

2) an oligonucleotide is used to replace sequences from IL-3 and mIL-6 which are lost as a result of the excision of the IL-3 and mIL-6 portions of the genes from the plasmids. Replacement of the missing IL-3 and mIL-6 sequences by the oligonucleotide also serves to join the IL-3 and mIL-6 sequences together to form the chimeric IL-3/mIL-6 nucleic acid sequence in such a way that both interleukins are in frame for translation;

3) the chimeric IL-3/mIL-6 nucleic acid sequence is assembled by combining the IL-3 fragment, the mIL-6 fragment, and, optionally, the oligonucleotide into a plasmid. The plasmid contains a selectable marker, such as an antibiotic resistance gene.

4) the chimeric IL-3/mIL-6 sequence is amplified by, for example, PCR or cloning;

5) the amplified chimeric IL-3/mIL-6 sequence is inserted into an expression vector for xpression of the chimeric IL-3/mIL-6 protein. Preferably, a controllable protein expression system that causes the juxtaposition of a promoter to control the amino acid coding sequence as a non-fusion process is employed. The system can utilize any of several well-known, characterized and available promoters such as *trp*, *trc*, *tic*, *tac*, *lac*, P_L, etc.

6) following expression of the chimeric IL-3/mIL-6 protein, the chimera is isolated and purified by methods known in the art.

The starting materials for construction of the present invention are nucleic acid sequences that encode native IL-3 and either native IL-6 or mIL-6. Nucleic acid sequences encoding native IL-3 and IL-6 may be isolated from a human cDNA or genomic DNA library.

The preferred method for obtaining DNA suitable as a starting material for construction of DNA encoding the chimera of the invention is to isolate DNA encoding native IL-3 and mIL-6 from an available recombinant plasmid. Recombinant plasmids that encode native full length IL-3 and mIL-6 are known. For IL-3, see, for example, PCT publication WO 88/00598, published 28 January 1988 and PCT publication 92/04455, published 19 March 1992. For mIL-6, see, for example, Skelly et al., U.S. application 07/907,710; Dagan et al., *Protein Expression and Purification* 3, 290-294 (1992); and Snouwaert, J., et al., *J. Immunol.* 146, 585-591 (1991).

If native IL-6 DNA is used as a starting material, mIL-6 is produced by mutating the native sequence. For example, muteins may be introduced into native IL-6 by site-directed mutagenesis, in order to encode amino acid residues other than cysteine at amino acid positions 45 and 51. Site-directed

mutagenesis is carried out by methods known in the art. See, for example, Zoller and Smith, Nucl. Acids Res. 10, 6487-6500 (1982); Methods in Enzymology 100, 468-500 (1983); and DNA 3, 479-488 (1984).

Recombinant plasmids that encode native IL-6 containing the four cysteine residues are known; see, for example, Clark et al., PCT application WO88/00206; Brakenhoff et al., Journal of Immunology 143, 1175-1182 (1989); Brakenhoff et al., Journal of Immunology 139, 4116-4121 (1987); Hirano et al., Proc. Natl. Acad. Sci. USA 84, 228-231 (1987). The codons for the cysteine residues at positions corresponding to positions 45 and 51 of native IL-6 are replaced by codons for other amino acids, preferably by codons for any other neutral amino acids, and more preferably by codons for serine or alanine residues.

Alternatively, plasmids containing DNA that encodes variants of native IL-6 in which all four cysteine residues have been replaced by serine residues may be obtained as described in Fowlkes et al., PCT application US89/05421. The codons for the serine residues at positions corresponding to positions 74 and 84 of native IL-6 are replaced by cysteine residues by, for example, site-directed mutagenesis. The codons for the serine residues at positions corresponding to 45 and 51 may be retained or replaced by other amino acid residues, such as by alanine, in the same way.

As an alternative, DNA encoding IL-3, IL-6, mIL-6 or the IL-3/mIL-6 chimera may be synthesized from individual nucleotides. Chemical synthesis of DNA from the four nucleotides may be accomplished in whole or in part by methods known in the art. Such methods include those described by Caruthers in Science 230, 281-285 (1985). DNA may also be synthesized by preparing

overlapping double-stranded oligonucleotides, filling in the gaps, and ligating the ends together.

Construction of the chimeric DNA sequences that encode the protein of the present invention is described below in Example 1.

The DNA obtained may be amplified by methods known in the art. One suitable method is the polymerase chain reaction (PCR) method described by Saiki et al. in Science 239, 487 (1988), Mullis et al in U.S. Patent 4,683,195 and by Sambrook, Fritsch and Maniatis (eds) in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989). It is convenient to amplify the clones in the lambda-gt10 or lambda-gt11 vectors using lambda-gt10 or lambda-gt11-specific oligomers as the amplimers (available from Clontech, Palo Alto, California).

The DNA fragments encoding the protein of the invention may be assembled in the proper order and replicated following insertion into a wide variety of host cells in a wide variety of cloning vectors. The host may be prokaryotic or eukaryotic.

Cloning vectors may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from *E.coli*, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13 fd, and other filamentous single-stranded DNA phages.

Vectors for expressing proteins in bacteria, especially *E.coli*, are also known. Such vectors include the pK233 (or any of the *tac* family of plasmids), T7, and lambda P_L. Examples of vectors that express fusion proteins are PATH

vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). Thes vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); maltose binding protein (pMAL); glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful for cloning and expression in yeast are available. A suitable example is the 2m circle plasmid.

Suitable cloning/expression vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, cytomegalovirus (CMV) retrovirus-derived DNA sequences. Any such vectors, when coupled with vectors derived from a combination of plasmids and phage DNA, i.e. shuttle vectors, allow for the isolation and identification of protein coding sequences in prokaryotes.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The expression v ctors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

Useful expression hosts include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCI, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*. Suitable eukaryotic cells include yeasts and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

The chimeric protein of the invention may be expressed in the form of a fusion protein with an appropriate fusion partner. The fusion partner preferably facilitates purification and identification. Increased yields may be achieved when the fusion partner is expressed naturally in the host cell. Some useful fusion partners include beta-galactosidase (Gray, et al., Proc. Natl. Acad. Sci. USA 79, 6598 (1982)); trpE (Itakura et al., Science 198, 1056 (1977)); protein A (Uhlen et al., Gene 23 369 (1983)); glutathione S-transferase (Johnson, Nature

338, 585 (1989)); Van Etten et al., Cell 58, 669 (1989)); and maltose binding protein (Guan et al., Gene 67, 21-30 (1987); Maina et al., Gene 74, 36-373 (1988); Riggs, P., in Ausebel, F.M. et al (eds) Current Protocols in Molecular Biology, Greene Associates/Wiley Interscience, New York (1990)).

Such fusion proteins may be purified by affinity chromatography using reagents that bind to the fusion partner. The reagent may be a specific ligand of the fusion partner or an antibody, preferably a monoclonal antibody. For example, fusion proteins containing beta-galactosidase may be purified by affinity chromatography using an anti-beta-galactosidase antibody column (Ullman, Gene. 29, 27-31 (1984)). Similarly, fusion proteins containing maltose binding protein may be purified by affinity chromatography using a column containing cross-linked amylose; see Guan, European Patent Application 286,239.

The fusion protein may occur at the amino-terminal or the carboxy-terminal side of the cleavage site. Optionally, the DNA that encodes the fusion protein is engineered so that the fusion protein contains a cleavable site between the protein and the fusion partner. Both chemical and enzymatic cleavable sites are known in the art. Suitable examples of sites that are cleavable enzymatically include sites that are specifically recognized and cleaved by collagenase (Keil et al., FEBS Letters 56, 292-296 (1975)); enterokinase (Hopp et al., Biotechnology 6, 1204-1210 (1988)); factor Xa (Nagai et al., Methods Enzymol. 153, 461-481 (1987)); and thrombin (Eaton et al., Biochemistry 25, 505 (1986)). Collagenase cleaves between proline and X in the sequence Pro-X-Gly-Pro wherein X is a neutral amino acid. Enterkinase cleaves after lysine in the sequence Asp-Asp-Asp-Asp-Lys. Factor Xa cleaves after arginine in the sequence Ile-Glu-Gly-Arg. Thrombin cleaves between arginine and glycine in the sequence Arg-Gly-Ser-Pro.

Specific chemical cleavage agents are also known. For example, cyanogen bromide cleaves at methionine residues in proteins.

The chimeric protein is purified by methods known in the art. Such methods include affinity chromatography using specific antibodies. Alternatively, the recombinant protein may be purified using a combination of ion-exchange, size-exclusion, and hydrophobic interaction chromatography using methods known in the art. These and other suitable methods are described by Marston, "The Purification of Eukaryotic Proteins Expressed in E. coli" in DNA Cloning, D. M. Glover, Ed., Volume III, IRL Press Ltd., England, 1987.

SEQ. ID. NOS. 1-2 show the amino acid sequence of one chimeric IL-3/mIL-6 protein of the invention. This sequence shows an embodiment in which the carboxy terminal end of IL-3 is attached to the amino terminal end of mIL-6. A nucleotide sequence that expresses the chimer is also shown in SEQ. ID. NO. 1.

In addition another mutein of IL-6 which has increased activity resulting from an amino acid substitution at, or corresponding to, amino acid location 171 or 175 of IL-6 having the wild-type sequence has been described by Leebeek, F.W.G., et al., *J. Biol. Chem.* 267 (21) 14832-14838 (1992). Substitutions of these carboxy-terminal amino acids may be introduced into the mIL-6 portion of the chimer of the present invention.

The invention also includes equivalent variants of the IL-3 and mIL-6 portions of the chimeric protein described above and the nucleic acid molecules that encode such variants. Equivalent variants include proteins comprising

substitutions and additions in the amino acid and nucleotide sequences of the chimeras of the invention and the corresponding nucleic acid molecules. Variants are included in the invention as long as the resulting chimeras and nucleic acid molecules continue to satisfy the structural and functional criteria described above, i.e., retain activity at least comparable to that of native IL-3 and mIL-6 and lack cysteine residues at positions 45 and 51 of the IL-6 portion. An amino acid or nucleotide sequence that is substantially the same as another sequence, but that differs from the other sequence by means of one or more substitutions or additions is considered to be an equivalent sequence. Except for the substitutions of cysteine residues at positions corresponding to positions 45 and 51 of native, mature IL-6, preferably less than 25%, more preferably less than 10%, and most preferably less than 5% of the total number of amino acids or nucleotides in the chimeras of the invention are substituted for or added to in the equivalent sequences.

For example, it is known to substitute amino acids in a sequence with equivalent amino acids. Groups of amino acids considered normally to be equivalent are:

(a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b) Asn(N) Asp(D) Glu(E) Gln(Q);
(c) His(H) Arg(R) Lys(K);
(d) Met(M) Leu(L) Ile(I) Val(V); and
(e) Phe(F) Tyr(Y) Trp(W).

Additions to the IL-3/mIL-6 muteins may be made at the C-terminal or N-terminal ends by adding the corresponding codons at the 5' or 3' ends of the nucleic acid sequences and expressing the nucleic acid molecules. Examples of internal additions to the nucleic acid molecules include the introns present in

genomic DNA. The introns are not expressed in a suitable eukaryotic host cell.

Equivalents of the nucleic acid molecules encoding the chimeric IL-3/mIL-6 protein also include silent mutations at sites that do not alter the amino acid sequence expressed. Preferably, the silent mutation results in increased expression in a particular host.

The chimera may contain the entire IL-3 and mIL-6 proteins, or a biologically active fragment of either or both whole proteins. Bioactive fragments of bioactive proteins may be identified by methods known in the art. For example, IL-6 fragments lacking amino acids 1-28 are known to be active. See, for example, Brakenhoff, J.P.J., et al., *J. Immunol.* 143, 1175-1182 (1989).

Fragments containing bioactive sequences may be selected on the basis of generally accepted criteria of potential bioactivity. Such criteria include analysis of which region(s) of a protein is required for bioactivity.

Methods for determining the biological activity of chimeric interleukin proteins are described in example 9 of PCT publication WO 92/04455, published 19 March 1992.

Nucleic Acid Molecules

The present invention includes nucleic acid molecules that encode the chimera of the present invention. Any nucleic acid sequence that encodes the amino acid sequence of SEQ. ID. NOS. 1-2 can be used to express the chimeric protein of the present invention. For example, nucleic acid sequences

that are found in nature or can be selected that will maximize expression in bacteria. The nucleic acid molecule may be DNA or RNA.

The nucleic acid molecules may be used as probes for detecting DNA encoding IL-3, IL-6, mIL-6 or chimeric IL-3/mIL-6 as explained below, or to produce a protein of the invention, as explained above.

Probes

The chimeric protein and DNA can be used to prepare probes that detect the presence of IL-3, IL-6, mIL-6 or the chimeric IL-3/mIL-6 protein or DNA in a sample. The method involves use of a labelled probe that recognizes IL-3, IL-6, mIL-6 or the chimeric IL-3/IL-6 protein or DNA present in biological samples, including, but not limited to, lymphatic fluid, synovial fluid, cerebral-spinal fluid, blood, tissue and cell samples. The probe may be an antibody raised against the chimeric IL-3/mIL-6 protein, or a fragment thereof, or an oligonucleotide that hybridizes to DNA encoding IL-3, IL-6, mIL-6 or the chimeric IL-3/mIL-6 protein. The antibody may be polyclonal or monoclonal.

Preparing Antibodies

Polyclonal antibodies are isolated from mammals that have been innoculated with the chimeric protein or a functional analog in accordance with methods known in the art. Briefly, polyclonal antibodies may be produced by injecting a host mammal, such as a rabbit, mouse, rat, or goat, with the chimeric protein or a fragment thereof. Sera from the mammal are extracted and screened to obtain polyclonal antibodies that are specific to the chimeric protein or protein fragment.

The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and by Campbell in "Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas" in Burdon et al., Eds, Laboratoty Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as the recombinant DNA method described by Huse et al. in Science 246, 1275-1281 (1989).

The probes described above are labelled in accordance with methods known in the art. The label may be a radioactive atom, an enzyme, or a chromophoric moiety.

Methods for labelling antibodies have been described, for example, by Hunter and Greenwood in Nature 144, 945 (1962) and by David et al. in Biochemistry 13, 1014-1021 (1974). Additional methods for labelling antibodies have been described in U.S. patents 3,940,475 and 3,645,090.

Methods for labelling oligonucleotide probes have been described, for example, by Leary et al., Proc. Natl. Acad. Sci. USA (1983) 80:4045; Renz and Kurz, Nucl. Acids Res. (1984) 12:3435; Richardson and Gumport, Nucl. Acids Res. (1983) 11:6167; Smith et al., Nucl. Acids Res. (1985) 13:2399; and Meinkoth and Wahl, Anal. Biochem. (1984) 138:267.

The label may be radioactive. Some examples of useful radioactive labels include ^{32}P, ^{125}I, ^{131}I, and ^{3}H. Use of radioactive labels have been described in U.K. 2,034,323, U.S. 4,358,535, and U.S. 4,302,204.

Som examples of non-radioactive labels include enzymes, chromophors, atoms and molecules detectable by electron microscopy, and metal ions detectable by their magnetic properties.

Detecting Protein with Antibodies

The probe may be an antibody, preferably a monoclonal antibody. The antibodies may be prepared as described above.

Assays for detecting the presence of proteins with antibodies have been previously described, and follow known formats, such as standard blot and ELISA formats. These formats are normally based on incubating an antibody with a sample suspected of containing the protein and detecting the presence of a complex between the antibody and the protein. The antibody is labelled either before, during, or after the incubation step. The protein is preferably immobilized prior to detection. Immobilization may be accomplished by directly binding the protein to a solid surface, such as a microtiter well, or by binding the protein to immobilized antibodies.

In a preferred embodiment, a protein is immobilized on a solid support through an immobilized first antibody specific for the protein. The immobilized first antibody is incubated with a sample suspected of containing the protein. If present, the protein binds to the first antibody.

A second antibody, also specific for the protein, binds to the immobilized protein. The second antibody may be labelled by methods known in the art. Non-immobilized materials are washed away, and the presence of immobilized label indicates the presence of the protein. This and other immunoassays are

described by David, et al. in U.S. Patent 4,376,110 assigned to Hybritech, Inc., LaJolla, California.

Detecting Antibodies with Protein

The chimeric protein may be labelled and used as probes in standard immunoassays to detect antibodies against IL-3, IL-6, mIL-6 or chimeric IL-3/mIL-6 proteins in samples, such as in the sera or other bodily fluids of patients. In general, a protein in accordance with the invention is incubated with the sample suspected of containing antibodies to the protein. The protein is labelled either before, during, or after incubation. The detection of labelled protein bound to an antibody in the sample indicates the presence of the antibody. The antibody is preferably immobilized.

Suitable assays are known in the art, such as the standard ELISA protocol described by R.H. Kenneth, "Enzyme-Linked Antibody Assay with Cells Attached to Polyvinyl Chloride Plates" in Kenneth et al, Monoclonal Antibodies, Plenum Press, N.Y., page 376 (1981).

Oligonucleotide Probes

The probe may also be an oligonucleotide complementary to a target nucleic acid molecule. The nucleic acid molecules may be RNA or DNA.

The length of the oligonucleotide probe is not critical, as long as it is capable of hybridizing to the target molecule. The oligonucleotide should contain at least 6 nucleotides, preferably at least 10 nucleotides, and, more preferably, at least 15 nucleotides.

There is no upper limit to the length of the oligonucleotide probes. Longer probes are more difficult to prepare and require longer hybridization times. Therefore, the probe should not be longer than necessary. Normally, the oligonucleotide probe will not contain more than 50 nucleotides, preferably not more than 40 nucleotides, and, more preferably, not more than 30 nucleotid s.

The chimeric IL-3/mIL-6 protein of the present invention possesses *in vitro* and *in vivo* biological activity at least comparable to that of a mixture of IL-3 and IL-6 or IL-3 and mIL-6. Accordingly, the chimeric IL-3/mIL-6 protein is useful in the *in vitro* and *in vivo* stimulation of the formation, proliferation and differentiation of a broad range of hematopoietic cells, including granulocytes, macrophages, eosinophils, mast cells, erythroid cells, B cells, T cells, megakaryocytes, and multi-potential hematopoietic progenitor cells. The stimulation of proliferation of megakaryocytes leads to the production of platelets. In addition, the mIL-6 portion of the chimeric IL-3/mIL-6 protein induces various acute phase proteins in liver cells. As a result of these biological activities, the chimeric IL-3/mIL-6 protein is useful in immunotherapeutic and anti-inflammation compositions. The chimera may also be used for the treatment of patients suffering from thrombocytopenia and patients undergoing chemotherapy or bone marrow transfers.

EXAMPLES

Example 1.

A. Construction of the Chimeric IL-3/mIL-6 Nucleic Acid Sequence.

The starting material for the construction of the chimeric IL-3/mIL-6 nucleic acid sequence is a plasmid, designated p570 (ATCC 69242). The p570

plasmid contains the cloned mature human IL-3 gene. An analogous plasmid containing sequences that encode mature human IL-3 can be obtained from R&D Systems Inc., Minneapolis, Mn., catalog No. BBG 14. Mature human IL-3 contains 133 amino acids. (See line A in Figure 1 and SEQ. ID. NO. 3-4)

The p570 plasmid is digested with the restriction endonucleases *Nco*I and *Dde*I. (New England Bio Labs, Beverly, Ma.) Digestion of the plasmid with these enzymes liberates a 0.375 kbp fragment (Line B in Figure 1) which encodes the natural amino terminus of human IL-3 and extends toward the carboxy terminus of the protein to the codon encoding alanine at amino acid position number 121. (See SEQ. ID. NO. 5)

The mIL-6 nucleic acid sequences are obtained from a plasmid designated pKK233-2 IL-6 SSCC. (See SEQ. ID. NO. 6-7 for the portion of the plasmid the encodes the sequence of mIL-6) Construction of the plasmid is described by Skelly et al., in example 5 of co-pending U.S. application 07/907,710, which is incorporated herein by reference and in Dagan et al., *Protein Expression and Purification* 3, 290-294 (1992). The pKK233-2 IL-6 SSCC plasmid contains a 0.6 kbp *Nco*I/*Hind*III restriction fragment that encodes mature mIL-6. The *Nco*I restriction site of this plasmid places an ATG codon immediately upstream of the initial mIL-6 amino acid residue, alanine. The *Nco*I site is followed 12 bp downstream by a unique *EcoR*II recognition sequence. As shown in Figure 1, when pKK233-2 IL-6 SSCC is digested with *EcoR*II and *Hind*III restriction enzymes (New England Bio Labs, Beverly, Ma.), a 0.59 kbp fragment is generated. (See line E and SEQ. ID. NO. 8) This fragment encodes the complete mIL-6 product minus the alanine-proline-valine-proline amino terminal residues and is followed by a *Kpn*I restriction site and three random in-frame stop codons.

Since the nucleic acid sequences encoding the last eleven amino acids from IL-3 and the first four amino acids from mIL-6 are lost as a result of the restriction endonuclease excision of the genes from their respective plasmids, an oligonucleotide pair (lines D and D' in Figure 1) encoding the lost amino acids is used to replace the lost nucleic acid sequences. In addition to replacing the lost nucleic acid sequences, the oligonucleotide pair (lines D and D' in Figure 1) join the IL-3 fragment (line B in Figure 1) to the mIL-6 fragment (line E in Figure 1) to form a chimeric IL-3/mIL-6 cassette with *Nco*I and *Hind*III termini. (See SEQ. ID. NO. 9) Synthesis of the oligonucleotides is described below in Section B. (See SEQ. ID. NOS. 10-11)

The chimeric IL-3/mIL-6 cassette is assembled by simultaneously combining the IL-3 fragment (component 1; line B in Figure 1), the mIL-6 fragment (component 2; line E in Figure 1) and the oligonucleotide pair (component 3; lines D and D' in Figure 1) with a plasmid (component 4) that has been pre-digested and purified by standard methods to remove a *Nco*I/*Hind*III restriction fragment from its sequence. The plasmid used in this example is designated pKK233-2 (Pharmacia LKB, Piscataway, N.J.). Once assembled, the chimeric IL-3/mIL-6 cassette, which has *Nco*I and *Hind*III termini, replaces the original *Nco*I/*Hind*III restriction fragment in the plasmid. The pKK233-2 plasmid contains an ampicillin resistance gene that is rendered functional if the four components of the reaction correctly assemble themselves to form the chimeric IL-3/mIL-6-pKK233-2 plasmid. The plasmid is transfected into *E.coli*. *E.coli* containing the chimeric IL-3/mIL-6 nucleic acid in the plasmid are selected for by growing the bacteria on agar containing ampicillin. Once selected, the IL-3/mIL-6-pKK233-2 plasmid is amplified to desired levels by growing the bacteria in a standard culture. (See Sambrook, Fritsch and Maniatis (eds) in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989)) The ampicillin-resistant clone is verified as

having the IL-3/mIL-6 gene by restriction enzyme analysis, sequencing data (Sanger, et al., 1977 Proc. Nat. Acad. of Sci., 74:5463) and expression of the IL-3/mIL-6 protein.

Expression of the IL-3/mIL-6 chimeric protein in *E.coli* is accomplished by inserting the chimeric IL-3/mIL-6 nucleic acid sequence into an expression vector. The expression vector pSE420 (*In Vitrogen*, San Diego, Ca.) contains the $lacI^q$ gene which allows for regulated expression in *E.coli* HB101. Transcriptional control is via the *trc* promoter and utilizes the highly efficient translation re-initiation characteristic of mini-cistron systems. The incorporation of upstream anti-termination and g10 ribosome binding sequences ensures high level translation of inserts cloned into its polylinker. Digestion of pSE420 with *Nco*I and *Kpn*I (New England Bio Labs, Beverly, Ma.) allows subsequent mobilization of the IL-3/mIL-6 chimera into this protein expression system by *Nco*I/*Kpn*I digestion of IL-3/mIL-6-pKK233-2 plasmid. (See SEQ. ID. NO. 12 for the sequence of the *Nco*I/*Kpn*I fragment) The resulting product is illustrated in Figures 2 and 3.

B. Synthesis of Oligonucleotides.

Oligonucleotide chains are specifically synthesized on a Model 392 Applied Biosystems apparatus utilizing beta-cyanoethyl phosphoramidites as substrate. Synthesized nucleotide oligomers are deprotected and cleaved from resin supports using standard procedures as recommended by the manufacturer. One may utilize any of a variety of oligonucleotide purification cartridges or proceed with HPLC purification and isolation.

C. Expression of Chimeric IL-3/mIL-6 Protein in *E.coli*.

Expression of the chimeric IL-3/mIL-6 protein is induced in high yield with isopropyl-beta-D-thiogalactopyranoside in E. coli strains HB101.

D. Purification of Chimeric IL-3/mIL-6 Protein.

Following expression of chimeric IL-3/mIL-6 protein in *E.coli*, the bacteria are harvested by centrifugation at 4°C and washed once in cold PBS. Bacterial pellets are suspended in 5ml/gm of cold 50mM Tris-HCl (pH 8.0), 100 mM NaCl, 1mM EDTA. Protease inhibitors PMSF (0.5mM), leupeptin (5mg/ml), aprotinin (5mg/ml) are included. Lysozyme, 50mg, is added and the suspension held on ice for 30 minutes. An equal volume of lysis buffer (50mm Tris-HCl, pH 8.0, 1% Triton X-100, 0.5% sodium deoxycholate) is added and the mixture gently rocked at room temperature for 30 minutes. $MgSO_4$ is added to a final concentration of 50mM followed by 25mg DNAaseI (New England Bio Labs, Beverly, Ma.). The mixture is incubated at room temperature until viscosity is minimal. This solution is then centrifuged at 10k rpm in a Beckman JS 13.1 swing-bucket rotor at 4°C. The pellet is washed once in Tris-HCl (pH 8.0), 100 mM NaCl and resuspended in this solution for protein determination by BioRad (Richmond, Ca.) assay.

Large Scale Chimera Purification

Frozen E. coli cell pellets (10g) are suspended in 50mM Tris-HCl pH 8.5, 5mM EDTA, 1mM AEBSF (buffer A). Lysozyme is added to a final concentration of 300mg/ml and the lysate is incubated on ice for 30 minutes. The lysate is homogenized on ice and then centrifuged at 10,000Xg for 30 minutes. The resulting pellet is washed 2X by centrifugation with buffer A containing 0.5% Triton X-100 and the supernatants discarded. The final pellet

containing chimeric IL-3/mIL-6 inclusion bodi s is resuspended in 50mM Tris-HCl pH 8.5, 6M guanidine-HCl, 1mM EDTA, 5mM DTT, 0.1mM AEBSF and incubated at room temperature for 2 hours. The extract is then clarified by centrifugation at 15,000Xg for 1hr.

The solubilized IL-3/mIL-6 is refolded by diluting the extract ten fold with 50mM Tris-HCl pH 8.5, 100mM NaCl, 1mM EDTA, 0.1mM AEBSF and incubating for 36hrs at 4°C. The protein concentration during refolding is < 0.2mg/ml. Insoluble material is removed by centrifugation and the supernatant dialyzed against 20mM Tris-HCl pH 8.5, 1mM EDTA, 0.1mM DTT.

Dialyzed IL-3/mIL-6 is applied to a Q-Sepharose HP (Pharmacia LKB, Piscataway, N.J.) anion exchange column (1.6 X 10cm) equilibrated in 20mM Tris-HCl pH 8.5 and eluted with a linear gradient of 500mM NaCl. Fractions containing the chimeric IL-3/mIL-6 are identified by ELISA, pooled and loaded onto a C4 reverse-phase column (Vydac C4, 4.6mm X 250mm) equilibrated in 100mM ammonium acetate (pH 6.0):isopropanol(85:15). The IL-3/mIL-6 is eluted with a linear gradient of 100mM ammonium acetate (pH 6.0):isopropanol (18:82) over 80 minutes at a flow rate of 0.7ml/min. Fractions containing purified IL-3/mIL-6 are pooled and stored at -70°C.

Final purity of the chimeric IL-3/mIL-6 is >90% as determined by silver stained SDS-PAGE gels. The final yield of purified IL-3/IL-6 from 10 grams of cell paste (wet weight) is ~350mg.

SUPPLEMENTAL ENABLEMENT

The invention as claimed is enabled in accordance with the specification and readily available references and starting materials.

Nevertheless, on February 8, 1993, Applicants have deposited with the American Type Culture Collection, Rockville, Md., USA (ATCC) the bacterial plasmid listed below: These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture for 30 years from date of deposit. The organisms will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Applicants and ATCC which assures unrestricted availability upon issuance of the pertinent U.S. patent. Availability of the deposited strains is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

NAME	Accession No.
p570	69242

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ImClone Systems Incorporated

(ii) TITLE OF INVENTION: Chimeric Interleukin-3/Interleukin-6 Lymphokine

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: ImClone Systems Incorporated
(B) STREET: 180 Varick Street
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10014

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Feit, Irving N.
(B) REGISTRATION NUMBER: 28,601
(C) REFERENCE/DOCKET NUMBER: TAC-4-T

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 212-645-1405
(B) TELEFAX: 212-645-2054

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 968 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..962

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 3..959

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CC ATG GCT CCG ATG ACC CAG ACC ACC TCC CTG AAA ACC TCC TGG GTT 47
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val
1 5 10 15

AAC TGT TCG AAC ATG ATC GAC GAA ATC ATC ACC CAC CTG AAA CAG CCG 95
Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro
20 25 30

CCG CTG CCG CTT CTA GAC TTC AAC AAC CTG AAC GGT GAA GAC CAG GAC 143
Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp
35 40 45

ATC CTG ATG GAA AAC AAC CTG CGT CGA CCG AAC CTG GAA GCA TTC AAC 191
Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn
50 55 60

CGT GCT GTT AAA AGC TTG CAG AAC GCT TCC GCT ATC GAA TCC ATC CTG 239

Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu
65 70 75

AAA AAC CTG CTG CCG TGC CTG CCG CTG GCT ACC GCG GCT CCG ACC CGT 287
Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg
80 85 90 95

CAC CCG ATC CAC ATC AAA GAC GGT GAC TGG AAC GAA TTT CGT CGT AAA 335
His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys
100 105 110

CTG ACC TTC TAC CTG AAA ACC CTC GAG AAC GCT CAG GCT CAG CAG ACC 383
Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr
115 120 125

ACC CTG TCC CTG GCT ATC TTC GCT CCG GTT CCG CCA GGA GAA GAT TCC 431
Thr Leu Ser Leu Ala Ile Phe Ala Pro Val Pro Pro Gly Glu Asp Ser
130 135 140

AAA GAT GTA GCC GCC CCA CAC AGA CAG CCG CTC ACC TCT TCA GAA CGA 479
Lys Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser Ser Glu Arg
145 150 155

ATC GAT AAA CAA ATT CGG TAC ATC CTC GAC GGG ATA TCA GCG CTG AGA 527
Ile Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg
160 165 170 175

AAA GAG ACC AGC AAC AAG AGT AAC ATG AGC GAA AGC AGT AAA GAA GCA 575
Lys Glu Thr Ser Asn Lys Ser Asn Met Ser Glu Ser Ser Lys Glu Ala
180 185 190

CTG GCA GAA AAC AAC CTG AAC CTT CCG AAG ATG GCT GAA AAA GAT GGA 623
Leu Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu Lys Asp Gly
195 200 205

TGT TTT CAA TCT GGA TTC AAT GAG GAA ACT TGT CTG GTG AAA ATC ATC 671
Cys Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys Ile Ile
210 215 220

ACA GGC CTT TTG GAA TTT GAG GTA TAC CTA GAG TAC CTC CAG AAC AGA 719
Thr Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln Asn Arg
225 230 235

TTT GAG AGT AGT GAG GAA CAA GCG AGA GCT GTC CAG ATG TCG ACC AAA 767
Phe Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met Ser Thr Lys
240 245 250 255

GTC CTG ATC CAG TTT CTG CAG AAA AAG GCA AAA AAT CTA GAT GCA ATA 815
Val Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp Ala Ile
260 265 270

ACC ACC CCG GAT CCA ACC ACA AAT GCG AGC CTG CTG ACG AAG CTG CAG 863
Thr Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln
275 280 285

GCA CAG AAC CAG TGG CTG CAG GAC ATG ACA ACT CAT CTC ATT CTG AGA 911
Ala Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile Leu Arg
290 295 300

TCT TTC AAA GAA TTC CTG CAG TCC TCC CTG CGT GCT CTG CGT CAG ATG 959
Ser Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln Met
305 310 315

TAATGATAG 968

320

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 319 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro
20 25 30

Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile
35 40 45

Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg
50 55 60

Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys
65 70 75 80

Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His
85 90 95

Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu
100 105 110

Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr
115 120 125

Leu Ser Leu Ala Ile Phe Ala Pro Val Pro Pro Gly Glu Asp Ser Lys
130 135 140

Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile
145 150 155 160

Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys
165 170 175

Glu Thr Ser Asn Lys Ser Asn Met Ser Glu Ser Ser Lys Glu Ala Leu
180 185 190

Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu Lys Asp Gly Cys

195 200 205

Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys Ile Ile Thr
210 215 220

Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe
225 230 235 240

Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met Ser Thr Lys Val
245 250 255

Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr
260 265 270

Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala
275 280 285

Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile Leu Arg Ser
290 295 300

Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln Met
305 310 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 404 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 3..404

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..404

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CC ATG GCT CCG ATG ACC CAG ACC ACC TCC CTG AAA ACC TCC TGG GTT 47
Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val
1 5 10 15

AAC TGT TCG AAC ATG ATC GAC GAA ATC ATC ACC CAC CTG AAA CAG CCG 95
Asn Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro
20 25 30

CCG CTG CCG CTT CTA GAC TTC AAC AAC CTG AAC GGT GAA GAC CAG GAC 143
Pro Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp
35 40 45

ATC CTG ATG GAA AAC AAC CTG CGT CGA CCG AAC CTG GAA GCA TTC AAC 191
Ile Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn
50 55 60

CGT GCT GTT AAA AGC TTG CAG AAC GCT TCC GCT ATC GAA TCC ATC CTG 239
Arg Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu
65 70 75

AAA AAC CTG CTG CCG TGC CTG CCG CTG GCT ACC GCG GCT CCG ACC CGT 287
Lys Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg
80 85 90 95

CAC CCG ATC CAC ATC AAA GAC GGT GAC TGG AAC GAA TTT CGT CGT AAA 335
His Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys
100 105 110

CTG ACC TTC TAC CTG AAA ACC CTC GAG AAC GCT CAG GCT CAG CAG ACC 383
Leu Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr
115 120 125

ACC CTG TCC CTG GCT ATC TTC 404
Thr Leu Ser Leu Ala Ile Phe
130

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15

Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro
20 25 30

Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile
35 40 45

Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn Arg
50 55 60

Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys
65 70 75 80

Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His
85 90 95

Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu
100 105 110

Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr
115 120 125

Leu Ser Leu Ala Ile Phe
130

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 378 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATGGCTCC GATGACCCAG ACCACCTCCC TTTTTTGAAA
ACCTCCTGGG TTAACTGTTC 60

GAACATGATC GACGAAATCA TCACCCACCT GAAACAGCCG
CCGCTGCCGC TTCTAGACTT 120

CAACAACCTG AACGGTGAAG ACCAGGACAT CCTGATGGAA
AACAACCTGC GTCGACCGAA 180

CCTGGAAGCA TTCAACCGTG CTGTTAAAAG CTTGCAGAAC
GCTTCCGCTA TCGAATCCAT 240

CCTGAAAAAC CTGCTGCCGT GCCTGCCGCT GGCTACCGCG
GCTCCGACCC GTCACCCGAT 300

CCACATCAAA GACGGTGACT GGAACGAATT TCGTCGTAAA
CTGACCTTCT ACCTGAAAAC 360

CCTCGAGAAC GCTCAGGC 378

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 564 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xl) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCTCCGGTTC CGCCAGGAGA AGATTCCAAA GATGTAGCCG
CCCCACACAG ACAGCCGCTC 60

ACCTCTTCAG AACGAATCGA TAAACAAATT CGGTACATCC
TCGACGGGAT ATCAGCGCTG 120

AGAAAAGAGA CCAGCAACAA GAGTAACATG AGCGAAAGCA
GTAAAGAAGC ACTGGCAGAA 180

AACAACCTGA ACCTTCCGAA GATGGCTGAA AAAGATGGAT GTTTTCAATC
TGGATTCAAT 240

GAGGAAACTT GTCTGGTGAA AATCATCACA GGCCTTTTGG
AATTTGAGGT ATACCTAGAG 300

TACCTCCAGA ACAGATTTGA GAGTAGTGAG GAACAAGCGA
GAGCTGTCCA GATGTCGACC 360

AAAGTCCTGA TCCAGTTTCT GCAGAAAAAG GCAAAAAATC TAGATGCAAT
AACCACCCCG 420

GATCCAACCA CAAATGCGAG CCTGCTGACG AAGCTGCAGG
CACAGAACCA GTGGCTGCAG 480

GACATGACAA CTCATCTCAT TCTGAGATCT TTCAAAGAAT TCCTGCAGTC
CTCCCTGCGT 540

GCTCTGCGTC AGATGTAATG ATAG 564

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 585 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAGGAGAAG ATTCCAAAGA TGTAGCCGCC CCACACAGAC
AGCCGCTCAC CTCTTCAGAA 60

CGAATCGATA AACAAATTCG GTACATCCTC GACGGGATAT
CAGCGCTGAG AAAAGAGACC 120

AGCAACAAGA GTAACATGAG CGAAAGCAGT AAAGAAGCAC
TGGCAGAAAA CAACCTGAAC 180

CTTCCGAAGA TGGCTGAAAA AGATGGATGT TTTCAATCTG GATTCAATGA
GGAAACTTGT 240

CTGGTGAAAA TCATCACAGG CCTTTTGGAA TTTGAGGTAT ACCTAGAGTA
CCTCCAGAAC 300

AGATTTGAGA GTAGTGAGGA ACAAGCGAGA GCTGTCCAGA
TGTCGACCAA AGTCCTGATC 360

CAGTTTCTGC AGAAAAAGGC AAAAAATCTA GATGCAATAA
CCACCCCGGA TCCAACCACA 420

AATGCGAGCC TGCTGACGAA GCTGCAGGCA CAGAACCAGT
GGCTGCAGGA CATGACAACT 480

CATCTCATTC TGAGATCTTT CAAAGAATTC CTGCAGTCCT CCCTGCGTGC
TCTGCGTCAG 540

ATGTAATGAT AGGTACCCGA GCTCGAATTC GTCGACCTGC AGCCA
585

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1006 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCATGGCTCC GATGACCCAG ACCACCTCCC TTTTTTGAAA
ACCTCCTGGG TTAACTGTTC 60

GAACATGATC GACGAAATCA TCACCCACCT GAAACAGCCG
CCGCTGCCGC TTCTAGACTT 120

CAACAACCTG AACGGTGAAG ACCAGGACAT CCTGATGGAA
AACAACCTGC GTCGACCGAA 180

CCTGGAAGCA TTCAACCGTG CTGTTAAAAG CTTGCAGAAC
GCTTCCGCTA TCGAATCCAT 240

CCTGAAAAAC CTGCTGCCGT GCCTGCCGCT GGCTACCGCG
GCTCCGACCC GTCACCCGAT 300

CCACATCAAA GACGGTGACT GGAACGAATT TCGTCGTAAA
CTGACCTTCT ACCTGAAAAC 360

CCTCGAGAAC GCTCAGGCTC AGCAGACCAC CCTGTCCCTG
GCTATCTTCG CTCCGGTTCC 420

GCCAGGAGAA GATTCCAAAG ATGTAGCCGC CCCACACAGA
CAGCCGCTCA CCTCTTCAGA 480

ACGAATCGAT AAACAAATTC GGTACATCCT CGACGGGATA
TCAGCGCTGA GAAAAGAGAC 540

CAGCAACAAG AGTAACATGA GCGAAAGCAG TAAAGAAGCA
CTGGCAGAAA ACAACCTGAA 600

CCTTCCGAAG ATGGCTGAAA AAGATGGATG TTTTCAATCT GGATTCAATG
AGGAAACTTG 660

TCTGGTGAAA ATCATCACAG GCCTTTTGGA ATTTGAGGTA TACCTAGAGT
ACCTCCAGAA 720

CAGATTTGAG AGTAGTGAGG AACAAGCGAG AGCTGTCCAG
ATGTCGACCA AAGTCCTGAT 780

CCAGTTTCTG CAGAAAAAGG CAAAAAATCT AGATGCAATA
ACCACCCCGG ATCCAACCAC 840

AAATGCGAGC CTGCTGACGA AGCTGCAGGC ACAGAACCAG
TGGCTGCAGG ACATGACAAC 900

TCATCTCATT CTGAGATCTT TCAAAGAATT CCTGCAGTCC TCCCTGCGTG
CTCTGCGTCA 960

GATGTAATGA TAGGTACCCG AGCTCGAATT CGTCGACCTG CAGCCA
1006

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCAGCAGACC ACCCTGTCCC TGGCTATCTT C 31

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAAGATAGCC AGGGACAGGG TGGTCTGCTG A 31

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
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(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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GAACATGATC GACGAAATCA TCACCCACCT GAAACAGCCG
CCGCTGCCGC TTCTAGACTT 120

CAACAACCTG AACGGTGAAG ACCAGGACAT CCTGATGGAA
AACAACCTGC GTCGACCGAA 180

CCTGGAAGCA TTCAACCGTG CTGTTAAAAG CTTGCAGAAC
GCTTCCGCTA TCGAATCCAT 240

CCTGAAAAAC CTGCTGCCGT GCCTGCCGCT GGCTACCGCG
GCTCCGACCC GTCACCCGAT 300

CCACATCAAA GACGGTGACT GGAACGAATT TCGTCGTAAA
CTGACCTTCT ACCTGAAAAC 360

CCTCGAGAAC GCTCAGGCTC AGCAGACCAC CCTGTCCCTG
GCTATCTTCG CTCCGGTTCC 420

GCCAGGAGAA GATTCCAAAG ATGTAGCCGC CCCACACAGA
CAGCCGCTCA CCTCTTCAGA 480

ACGAATCGAT AAACAAATTC GGTACATCCT CGACGGGATA
TCAGCGCTGA GAAAAGAGAC 540

CAGCAACAAG AGTAACATGA GCGAAAGCAG TAAAGAAGCA
CTGGCAGAAA ACAACCTGAA 600

CCTTCCGAAG ATGGCTGAAA AAGATGGATG TTTTCAATCT GGATTCAATG
AGGAAACTTG 660

TCTGGTGAAA ATCATCACAG GCCTTTTGGA ATTTGAGGTA TACCTAGAGT
ACCTCCAGAA 720

CAGATTTGAG AGTAGTGAGG AACAAGCGAG AGCTGTCCAG
ATGTCGACCA AAGTCCTGAT 780

CCAGTTTCTG CAGAAAAAGG CAAAAAATCT AGATGCAATA
ACCACCCCGG ATCCAACCAC 840

AAATGCGAGC CTGCTGACGA AGCTGCAGGC ACAGAACCAG
TGGCTGCAGG ACATGACAAC 900

TCATCTCATT CTGAGATCTT TCAAAGAATT CCTGCAGTCC TCCCTGCGTG
CTCTGCGTCA 960

GATGTAATGA TAGGTAC 977

CLAIMS

What is claimed is:

1. A chimeric protein comprising:

an amino portion having the amino acid sequence of interleukin-3 and a carboxy portion having the amino acid sequence of mutein interleukin-6.

2. The chimeric protein of claim 1, wherein the biological activity of the interleukin-3 portion of the chimeric protein is at least comparable to the biological activity of native interleukin-3.

3. The chimeric protein of claim 1, wherein the biological activity of the mutein interleukin-6 portion of the chimeric protein is at least comparable to the biological activity of mutein interleukin-6.

4. The chimeric protein of claim 1, wherein the biological activity of the interleukin-3 portion of the chimeric protein is at least comparable to the biological activity of native interleukin-3 and the biological activity of the mutein interleukin-6 portion of the chimeric protein is at least comparable to the biological activity of mutein interleukin-6.

5. The chimeric protein of claim 1, wherein the interleukin-3 portion is of human origin.

6. The chimeric protein of claim 1, wherein the mutein interleukin-6 portion is derived from native IL-6 of human origin.

7. The chimeric protein of claim 1, wherein the interleukin-3 portion is of human origin and the mutein interleukin-6 portion is derived from native IL-6 of human origin.

8. A chimeric protein according to the claim 1 wherein the amino acid sequence is:

Met Ala Pro Met Thr Gln Thr Thr Ser Leu Lys Thr Ser Trp Val Asn
1 5 10 15
Cys Ser Asn Met Ile Asp Glu Ile Ile Thr His Leu Lys Gln Pro Pro
20 25 30
Leu Pro Leu Leu Asp Phe Asn Asn Leu Asn Gly Glu Asp Gln Asp Ile
35 40 45
Leu Met Glu Asn Asn Leu Arg Arg Pro Asn Leu Glu Ala Phe Asn
Arg 50 55 60
Ala Val Lys Ser Leu Gln Asn Ala Ser Ala Ile Glu Ser Ile Leu Lys 65
70 75 80
Asn Leu Leu Pro Cys Leu Pro Leu Ala Thr Ala Ala Pro Thr Arg His
85 90 95
Pro Ile His Ile Lys Asp Gly Asp Trp Asn Glu Phe Arg Arg Lys Leu
100 105 110
Thr Phe Tyr Leu Lys Thr Leu Glu Asn Ala Gln Ala Gln Gln Thr Thr
115 120 125
Leu Ser Leu Ala Ile Phe Ala Pro Val Pro Pro Gly Glu Asp Ser Lys
130 135 140
Asp Val Ala Ala Pro His Arg Gln Pro Leu Thr Ser Ser Glu Arg Ile
145 150 155 160
Asp Lys Gln Ile Arg Tyr Ile Leu Asp Gly Ile Ser Ala Leu Arg Lys
165 170 175
Glu Thr Ser Asn Lys Ser Asn Met Ser Glu Ser Ser Lys Glu Ala Leu
180 185 190
Ala Glu Asn Asn Leu Asn Leu Pro Lys Met Ala Glu Lys Asp Gly Cys
195 200 205
Phe Gln Ser Gly Phe Asn Glu Glu Thr Cys Leu Val Lys Ile Ile Thr
210 215 220
Gly Leu Leu Glu Phe Glu Val Tyr Leu Glu Tyr Leu Gln Asn Arg Phe
225 230 235 240

Glu Ser Ser Glu Glu Gln Ala Arg Ala Val Gln Met Ser Thr Lys Val
245 250 255
Leu Ile Gln Phe Leu Gln Lys Lys Ala Lys Asn Leu Asp Ala Ile Thr
260 265 270
Thr Pro Asp Pro Thr Thr Asn Ala Ser Leu Leu Thr Lys Leu Gln Ala
275 280 285
Gln Asn Gln Trp Leu Gln Asp Met Thr Thr His Leu Ile Leu Arg Ser
290 295 300
Phe Lys Glu Phe Leu Gln Ser Ser Leu Arg Ala Leu Arg Gln Met
305 310 315

9. A nucleic acid molecule that encodes a chimeric protein wherein the chimeric protein comprises an amino portion having the amino acid sequence of interleukin-3 and a carboxy portion having the amino acid sequence of mutein interleukin-6.

10. The nucleic acid molecule of claim 9, wherein the biological activity of the interleukin-3 portion of the chimeric protein is at least comparable to the biological activity of native interleukin-3.

11. The nucleic acid molecule of claim 9, wherein the biological activity of the mutein interleukin-6 portion of the chimeric protein is at least comparable to the biological activity of mutein interleukin-6.

12. The nucleic acid molecule of claim 9, wherein the biological activity of the interleukin-3 portion of the chimeric protein is at least comparable to the biological activity of native interleukin-3 and the biological activity of the mutein

interleukin-6 portion of the chimeric protein is at least comparable to the biological activity of mutein interleukin-6.

13. The nucleic acid molecule of claim 9, wherein the interleukin-3 portion is of human origin.

14. The nucleic acid molecule of claim 9, wherein the mutein interleukin-6 portion is derived from native IL-6 of human origin.

15. The nucleic acid molecule of claim 9, wherein the interleukin-3 portion and the mutein interleukin-6 portion is derived from native IL-6 of human origin.

Figure 1

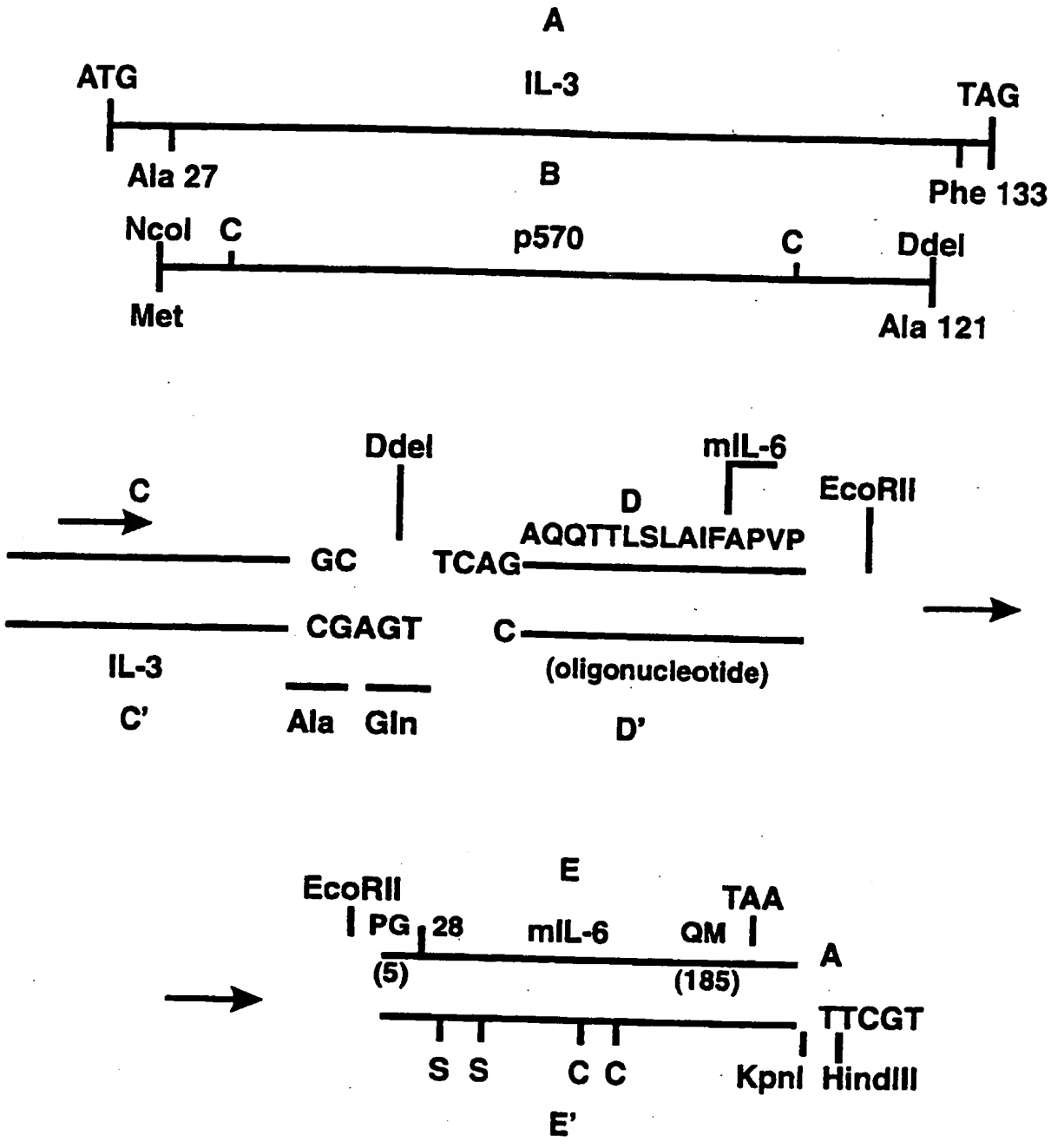

A
ATG
IL-3
TAG
Ala 27
B
Phe 133
NcoI C
p570
C
DdeI
Met
Ala 121
DdeI
mIL-6
C
D
EcoRII
AQQTTLSLAIFAPVP
GC
TCAG
CGAGT
C
IL-3
(oligonucleotide)
C'
Ala Gln
D'
E
EcoRII
TAA
PG 28
mIL-6
QM
A
(5)
(185)
TTCGT
S S
C C
KpnI HindIII
E'

Figure 2

Chimeric IL-3/mIL-6 Lymphokine

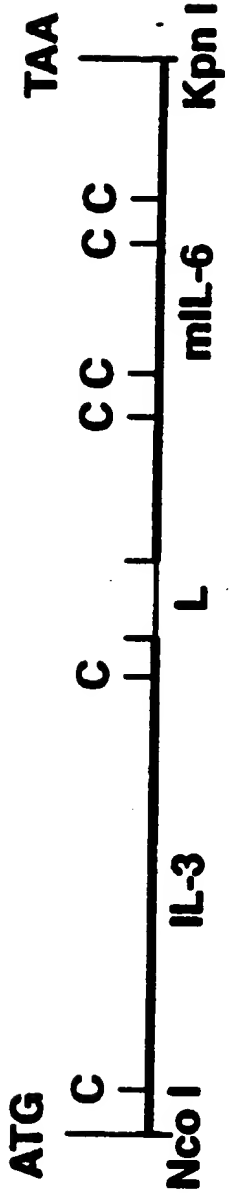
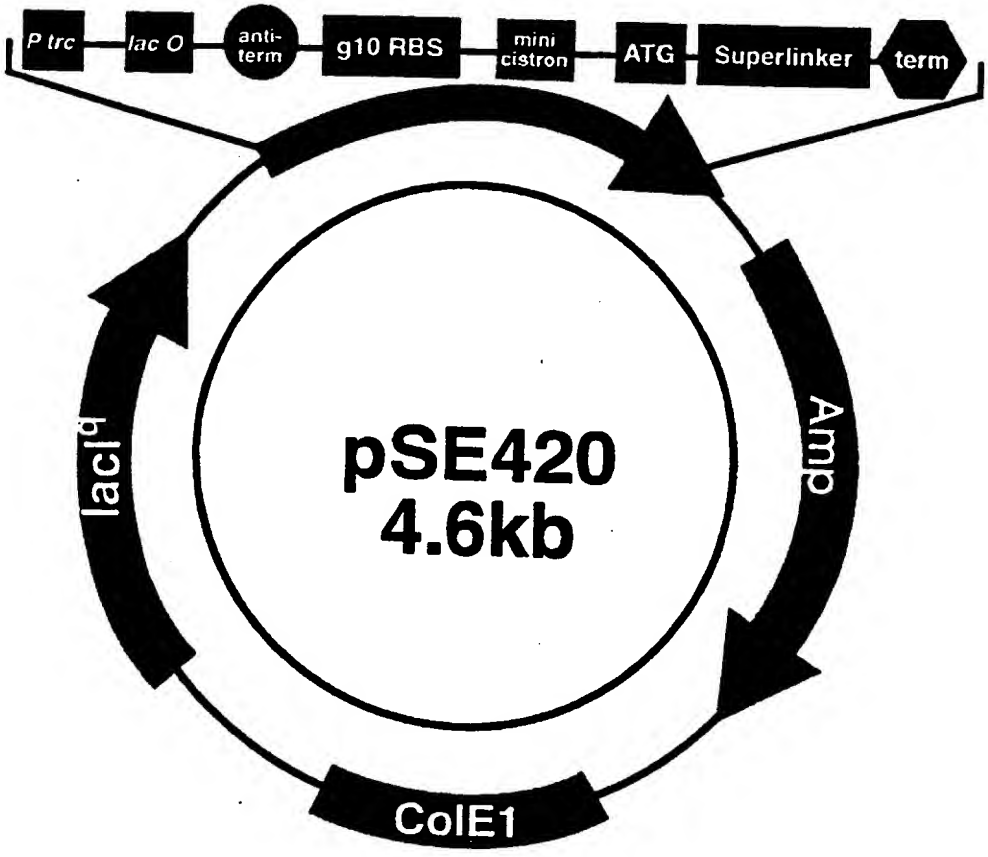


Figure 3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lac O
anti-term
g10 RBS
mini cistron
ATG
Superlinker
term
pSE420
4.6kb
lacI^q
Amp
ColE1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04208

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 15/00; C07H 15/12
US CL : 530/351, 402; 930/141, 142
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/351, 402; 930/141, 142

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GeneSeq, EMBL, GenBank, APS, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 5,114,711 (BELL ET AL.) 19 May 1992, see abstract.	1-15
Y	WO, A, 92/06116 (ROSEN) 16 April 1992, see the abstract, pages 6-8, and the claims.	1-15
Y	WO, A, 92/04455 (SCHENDEL) 19 March 1992, see the claims, pages 1-3, and example 7.	1-15
Y	Journal of Biological Chemistry, Volume 266, Number 34, issued 05 December 1991, Snouwaert et al., "Role of Disulfide Bonds in Biologic Activity of Human Interleukin-6", pages 23097-23102, see entire document.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
24 AUGUST 1994

Date of mailing of the international search report
SEP 20 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04208

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation f document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Experimental Medicine, Volume 170, issued August 1989, Bergui et al., "Interleukin 3 and Interleukin 6 Synergistically Promote the Proliferation and Differentiation of Malignant Plasma Cell Precursors in Multiple Myeloma", pages 613-618, see entire document.	1-15

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WO 9604388A1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/62, C07K 14/54, 16/00, 19/00, A61K 38/19, 39/395

A1

(11) International Publication Number: WO 96/04388

(43) International Publication Date: 15 February 1996 (15.02.96)

(21) International Application Number: PCT/EP95/03036

(22) International Filing Date: 28 July 1995 (28.07.95)

(30) Priority Data:

9415379.8	29 July 1994 (29.07.94)	GB
08/468,297	6 June 1995 (06.06.95)	US

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NOVEL COMPOUNDS

(57) Abstract

A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity comprises an IL4 mutant or variant fused to at least one human immunoglobulin constant domain or fragment thereof.

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NOVEL COMPOUNDS

The present invention relates to antagonists of human interleukin 4 (IL4) and/or human interleukin 13 (IL13) for the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as certain IgE mediated allergic diseases, T cell mediated autoimmune conditions and inappropriate immune responses to infectious agents.

Interleukins are secreted peptide mediators of the immune response. Each of the known interleukins has many effects on the development, activation, proliferation and differentiation of cells of the immune system. IL4 has a physiological role in such functions, but can also contribute to the pathogenesis of disease. In particular IL4 is associated with the pathway of B lymphocyte development that leads to the generation of IgE antibodies that are the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjunctivitis, atopic dermatitis and anaphylaxis. IL4 can also act as a general growth and differentiation factor for T lymphocytes that may contribute to tissue damage in certain autoimmune conditions such as insulin dependent diabetes, multiple sclerosis and rheumatoid arthritis and in graft rejection. IL4 can also suppress the generation of cell-mediated responses required for the control of infectious disease. Antagonism of the effect of IL4 on T or B lymphocytes can therefore be expected to have beneficial effects on such diseases. IL13 has been recently identified and shares similarity in many of the biological properties of IL4 (Minty, A. *et al* (1993), Nature **362**, 248-250) including some aspect(s) of receptor structure/function (Aversa, G. *et al* (1993), J. Exp. Med. **178**, 2213-2218).

Human IL4 consists of a single polypeptide chain of 129 amino acids with 2 possible N-glycosylation sites and 6 cysteines involved in 3 disulphide bridges (Le, H.V. *et. al.*, (1988), J. Biol. Chem. **263**, 10817-10823). The amino acid sequence of IL4 and the positions of these disulphide bridges are known (Carr, C. *et al.*, (1991) Biochemistry **30**, 1515-1523).

```
                                        10
HIS-LYS-CYS-ASP-ILE-THR-LEU-GLN-GLU-ILE-ILE-LYS-THR-LEU-ASN-

                    20                                      30
SER-LEU-THR-GLU-GLN-LYS-THR-LEU-CYS-THR-GLU-LEU-THR-VAL-THR-

                                        40
ASP-ILE-PHE-ALA-ALA-SER-LYS-ASN-THR-THR-GLU-LYS-GLU-THR-PHE-

                    50                                      60
CYS-ARG-ALA-ALA-THR-VAL-LEU-ARG-GLN-PHE-TYR-SER-HIS-HIS-GLU-

                                    70
LYS-ASP-THR-ARG-CYS-LEU-GLY-ALA-THR-ALA-GLN-GLN-PHE-HIS-ARG-

                    80                                      90
```

```
HIS-LYS-GLN-LEU-ILE-ARG-PHE-LEU-LYS-ARG-LEU-ASP-ARG-ASN-LEU-

                                        100
TRP-GLY-LEU-ALA-GLY-LEU-ASN-SER-CYS-PRO-VAL-LYS-GLU-ALA-ASN-

                  110                                       120
GLN-SER-THR-LEU-GLU-ASN-PHE-LEU-GLU-ARG-LEU-LYS-THR-ILE-MET-

                                    129
ARG-GLU-LYS-TYR-SER-LYS-CYS-SER-SER
```

The disulphide bridges are between residues 3 and 127, 24 and 65, and 46 and 99. The molecular weight of IL4 varies with the extent of glycosylation from 15KDa (no glycosylation) to 60KDa or more (hyperglycosylated IL4).

The DNA sequence for human IL4 has also been described by Yokota, T. *et. al.*, P.N.A.S. 1986 **83** 5894-5898.

WO 93/10235 describes certain mutants of IL4 which are IL4 antagonists or partial antagonists.

EP-A-0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof.

The present invention provides a soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity, comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.

The term "mutant or variant" encompasses any molecule such as a truncated or other derivative of the IL4 protein which retains the ability to antagonise IL4 and/or IL13 following internal administration to a human. Such other derivatives can be prepared by the addition, deletion, substitution, or rearrangement of amino acids or by chemical modifications thereof.

DNA polymers which encode mutants or variants of IL4 may be prepared by site-directed mutagenesis of the cDNA which codes for IL4 by conventional methods such as those described by G. Winter *et al* in Nature 1982, **299**, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., **10**, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, **12**, 2407-2419 or by G. Winter *et al* in Biochem. Soc. Trans., 1984; **12**, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, **20**, 376.

As used herein, "having IL4 and/or IL13 antagonist or partial antagonist activity" means that, in the assay described by Spits *et al* (J. Immunology **139**, 1142 (1987)), IL4-stimulated T cell proliferation is inhibited in a dose-dependent manner.

Suitable IL4 mutants are disclosed in WO 93/10235, wherein at least one amino acid, naturally occuring in wild type IL4 at any one of positions 120 to 128

inclusive, is replaced by a different natural amino acid. In particular, the tyrosine naturally occurring at position 124 may be replaced by a different natural amino acid, such as glycine or, more preferably, aspartic acid.

The immunoglobulin may be of any subclass (IgG, IgM, IgA, IgE), but is preferably IgG, such as IgG1, IgG3 or IgG4. The said constant domain(s) or fragment thereof may be derived from the heavy or light chain or both. The invention encompasses mutations in the immunoglobulin component which eliminate undesirable properties of the native immunoglobulin, such as Fc receptor binding and/or introduce desirable properties such as stability. For example, Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule. Canfield S.M. and Morrison S.L., Journal of Experimental Medicine vol173pp1483-1491, describe the alteration of residue 248 (Kabat numbering) from leucine to glutamate in IgG3 and from glutamate to leucine in mouse IgG2b. Substitution of leucine for glutamate in the former decreases the affinity of the immunoglobulin molecule concerned for the Fcγ RI receptor, and substitution of glutamate for leucine in the latter increases the affinity. EP0307434 discloses various mutations including an L to E mutation at residue 248 (Kabat numbering) in IgG.

The constant domain(s) or fragment thereof is preferably the whole or a substantial part of the constant region of the heavy chain of human IgG, most preferably IgG4. In one aspect the IgG component consists of the CH2 and CH3 domains and the hinge region of IgG1 including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 11 and 14 of the IgG1 hinge region (Frangione B. and Milstein C., Nature vol216pp939-941, 1967). Preferably the IgG1 component consists of amino acids corresponding to residues 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG1 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. In another aspect the IgG component is derived from IgG4, comprising the CH2 and CH3 domains and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding, for example residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., Nature vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L., DNA vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10

of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

Fusion of the IL4 mutant or variant to the Ig constant domain or fragment is by C-terminus of one component to N-terminus of the other. Preferably the IL4 mutant or variant is fused via its C-terminus to the N-terminus of the Ig constant domain or fragment.

In a preferred aspect, the amino acid sequence of the fusion protein of the invention is represented by SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.

In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

In a preferred aspect the DNA polymer comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

In particular, the process may comprise the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and

iv) recovering said compound.

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts *et al* in Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical

synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less.

Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to ambient, generally in a volume of 50µl or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982),or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, **10**, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, **24**, 5771; M.D. Matteucci and M.H Caruthers, Tetrahedron Letters, 1980, **21**, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, **103**, 3185; S.P. Adams *et al.*, Journal of the American Chemical Society,1983, **105**, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, **12**, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, **3**, 801. Preferably an automated DNA synthesizer is employed.

The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the compound. A particular process in accordance with the invention comprises ligating a first DNA molecule encoding a said IL4 mutant or variant and a second DNA molecule encoding a said immunoglobulin domain or fragment thereof.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain reaction technology.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as Drosophila. The host cell may also be a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses, vaccinia or Semliki Forest virus.

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis et al., cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of $CaCl_2$ (Cohen *et al*, Proc. Nat. Acad. Sci., 1973, **69**, 2110) or with a solution comprising a mixture of RbCl, $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C.

The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. et al., Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*, European Patent Application No. 0093619, 1983).

Compounds of the present invention have IL4 and/or IL13 antagonist activity and are therefore of potential use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13 such as IgE mediated allergic diseases and T cell mediated autoimmune conditions or chronic microbial infection.

The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The compound may, for example, be employed in the form of aerosol or nebulisable solution for inhalation or sterile solutions for parenteral administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect on the IL4 and/or IL13 mediated condition, for example whereby IgE antibody mediated symptoms are reduced or progression of the autoimmune disease is halted or reversed. The dosage will generally vary with age, extent or severity of the medical condition and contraindications, if any. The unit dosage can vary from less than 1mg to 300mg, but

typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, such that the daily dosage is in the range 0.02-40mg/kg.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound of the invention.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating conditions resulting from undesirable actions of IL4 and/or IL13.

The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating conditions resulting from undesirable actions of IL4 and/or IL13.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The following Examples illustrate the invention.

Example 1 IL4.Y124D/IgG1 fusion protein

The construction of an IL4.Y124D/IgG1 chimeric cDNA, the expression of the corresponding protein in a mammalian expression system and its activity are described.

1. Construction of DNA coding for fusion protein

(a) Construction of IL4.Y124D coding region

A variant of the human IL4 gene, which has been described (Kruse, N, Tony, H-P and Sebald, W. EMBO Journal 11: 3237 [1992]) in which residue 124 in the protein has been mutated from tyrosine in the wild type to aspartic acid, was produced by PCR mutagenesis of the human IL4 cDNA (purchased from British Biotechnology). The IL4.Y124D cDNA was inserted into the expression vector pTR312, using the HindIII and BglII sites, (M J Browne, J E Carey, C G Chapman, A W R Tyrrell, C Entwisle, G M P Lawrence, B Reavy, I Dodd, A Esmail & J H Robinson. Journal of Biological Chemistry 263: 1599, [1988]) to form the plasmid pDB906.

To amplify the IL4.Y124D molecule and add convenient restriction sites at each end for subcloning, a PCR reaction was performed using 20ng of the pDB906 plasmid as the substrate. PCR primers were designed to include restriction enzyme sites, flanked by 10-15 nucleotide base pairs to "anchor" the primers at each end. The primer sequences were as follows:

1) 5' CGA ACC ACT GAA TTC CGC ATT GCA GAG ATA 3'
(includes an EcoRI restriction site, GAATTC)

2) 5' CAC AAA GAT CCT TAG GTA CCG CTC GAA CAC TTT GA 3'
(includes a KpnI restriction site, GGTACC)

Primers were used at a final concentration of 5ng/µl, and dNTPs were added at a final concentration of 0.2mM in a total reaction volume of 100µl. 31 cycles of PCR were performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes. 2.5 units of the Taq polymerase enzyme from Advanced Biotechnologies were used in the PCR reaction. A PCR product of 587bp was produced. This was purified using the Promega "Magic PCR cleanup" kit, and then digested with EcoRI and KpnI in react buffer 4 (all restriction enzymes were obtained from GibcoBRL.), to generate 'sticky ends'. After 4 hours 30 minutes at 37° C, the reaction was heated to 70°C for 10 minutes and then ethanol precipitated. Analysis of the resulting DNA by agarose gel electrophoresis showed the presence of three bands of approximately 570bp, 463bp and 100bp . The 570bp fragment represents the full-length IL4.Y124D variant of IL4 and was present because the digest was incomplete. The two smaller fragments were produced due to the presence of an EcoRI site within the IL4.Y124D cDNA. The 570bp band was purified by the Geneclean ™ procedure, and ligated into Bluescript KS$^+$ ™ which was prepared by digestion with EcoRI and KpnI followed by Geneclean ™. A Bluescript KS$^+$/IL4.Y124D recombinant was thus generated. Large amounts of this recombinant DNA were produced using the Promega "Magic Maxiprep" method. The IL4.Y124D insert was excised from the Bluescript recombinant using SmaI and KpnI. 20µg recombinant DNA was incubated with 25 units SmaI in react buffer 4, at 30°C overnight. 25 units of KpnI were then added to the digest, which was incubated at 37°C for 5 hours. The resulting fragment of approximately 580bp was purified by Geneclean ™ to generate an IL4.Y124D/SmaI/KpnI fragment.

(b) Construction of IgG1 coding region

The COSFcLink vector (Table 1) contains human IgG1 cDNA encoding amino acids 1-4 and 6-15 of the hinge, 1-110 of CH2 and 1-108 of CH3 described by Ellison J., Berson B. and Hood L. E., Nucleic Acids Research vol10, pp4071-4079, 1982. Residue 5 of the hinge is changed from cysteine in the published IgG1 sequence to alanine by alteration of TGT to GCC in the nucleotide sequence. This was cloned from the human IgG plasma cell leukemia ARH-77 (American Type Tissue Collection), using RT-PCR and fully sequenced to confirm identity with the published sequence [patent application publication WO 92/00985]

The construction of COSFc began with a pUC18 vector containing the human IgG1 cDNA above (pUC18-Fc), which was digested with KpnI and SacII, deleting the CH1, hinge and part of CH2. The deleted region was replaced with a PCR

amplified fragment containing the hinge-CH2 region as follows. Using the following PCR primers:

5' TCG AGC TCG GTA CCG AGC CCA AAT CGG CCG ACA AAA CTC ACA C 3'

and

5' GTA CTG CTC CTC CCG CGG CTT TGT CTT G 3'

A DNA fragment containing the hinge-CH2 region was amplified from pUC18-Fc, digested with KpnI and SacII, gel purified and cloned into the KpnI/SacII digested pUC18-Fc vector. The Cys, which occurs at position 230 (Kabat numbering; Kabat et al., "Sequences of Proteins of Immunological Interest, 5th Edition, US Department of Health and Human Services, NIH Publication No. 91-3242 (1991)) of the IgG1 heavy chain, was altered to an Ala through a TGT to GCC substitution in the nucleotide sequence. An altered DNA sequence in one of the PCR primers introduced a unique KpnI site at the 5' end of the hinge. The resulting plasmid was called pUC18Fcmod, and the junctions and PCR amplified region were sequenced for confirmation.

The entire hinge-CH2-CH3 insert in pUC18-Fcmod was removed in a single DNA fragment with KpnI and XbaI, gel purified, and ligated into SFcR1Cos4 cut with KpnI and XbaI to create COSFc.

SFcR1Cos4 is a derivative of pST4DHFR (Deen, K , McDougal, JS, Inacker, R, Folena-Wasserman, G, Arthos, J, Rosenberg, J, Maddon, PJ, Axel, R, and Sweet, RW. Nature 331: 82 [1988]) and contains the soluble Fc receptor type I (sFcR1) inserted between the cytomegalovirus (CMV) promoter and bovine growth hormone (BGH) polyadenylation regions, and also contains the dihydrofolate reductase (DHFR) cDNA inserted between the β-globin promoter and SV40 polyadenylation regions, an SV40 origin of replication, and an ampicillin resistance gene for growth in bacteria. Cutting the vector with KpnI and XbaI removes the sFcR1 coding region, so that the COSFc vector contains the hinge-CH2-CH3 region inserted between the CMV promoter and BGH polyA regions.

The COSFcLink vector was made from COSFc by inserting an oligonucleotide linker at the unique EcoRI site of the vector, which recreates this EcoRI site, and also introduces BstEII, PstI and EcoRV cloning sites. The oligonucleotides used were:

5' AATTCGGTTACCTGCAGATATCAAGCT 3'
3' GCCAATGGACGTCTATAGTTCGATTAA 5'

The junction was sequenced to confirm orientation in the vector. The size of the final vector is 6.37 kb.

(c) Construction of DNA coding for fusion protein.

To insert the IL4.Y124D cDNA, the COSFcLink vector was prepared by digesting with EcoRV and KpnI as follows: 5µg DNA was incubated with 15 units EcoRV in react 2 at 37°C for 5 hours, followed by ethanol precipitation. The resulting DNA was digested with KpnI in react 4 at 37°C for 3 hours, and ethanol precipitated. The IL4.Y124D/SmaI/KpnI and the COSFcLink/EcoRV/KpnI fragments were ligated together to form plasmid pDB951, which encodes the IL4.Y124D/IgG1 fusion protein. The ligation was achieved using an Amersham DNA ligation kit, product code RPN 1507, the reactions being incubated at 16°C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/COSFcLink recombinant DNA was verified by restriction digests and DNA sequencing. The complete IL4.Y124D sequence and the junctions with the COSFcLink DNA were confirmed by DNA sequencing (Table 2). The coding sequence of the recombinant IL4.Y124D/IgG1 DNA is shown in Table 3 and the amino acid sequence of the fusion protein is shown in Table 4. The IL4.Y124D/COSFcLink recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25µg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and

12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG1 chimera inhibited ^{3}H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 2 IL4.Y124D/IgG4 fusion protein

1. Construction of DNA coding for fusion protein

PCR was performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site. As substrate for the PCR reaction 20ng of linearised pDB951 plasmid (Example 1.1(c)) was used. The oligonucleotide primers used were as follows:

1) 5' CAC AAG TGC GAT ATC ACC TTA CAG GAG ATC 3'
(includes an EcoRV restriction site, GATATC)

2) 5' CTC GGT ACC GCT CGA GCA CTT TGA GTC TTT 3'
(includes a XhoI restriction site, CTCGAG).

A second PCR reaction was performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain. The substrate for this was a synthetic human IgG4 heavy chain cDNA, the sequence of which is described in Table 5, and is based on the Genbank sequence GB:HUMIGCD2 (Ellison J., Buxbaum J. and Hood L.E., DNA 1:11-18, 1981). Numerous silent substitutions were made to the published nucleotide sequence. The gene was assembled by combining two 0.5Kb synthetic DNA fragments. Each 0.5Kb fragment was made by annealing a series of

overlapping oligonucleotides and then filling in the gaps by PCR. The two 0.5Kb fragments were joined at the SacII site and inserted into the pCR2 vector. A 1.0Kb ApaI-BglII fragment containing the entire constant region was isolated and ligated into an expression vector, pCD, containing a humanized IL4 specific variable region. This construct was used as the PCR substrate to amplify the hinge-CH2-CH3 region of IgG4.

The oligonucleotide primers used for amplification of the IgG4 hinge-CH2-CH3 region were as follows:

1) 5' GGT GGA CAA CTC GAG CGA GTC CAA ATA TGG 3'
(includes a XhoI restriction site, CTCGAG)

2) 5' TTA CGT AGA TCT AGA CTA CAC TCA TTT ACC 3'
(includes an XbaI site, TCTAGA).

The conditions for both PCR reactions were as described for the derivation of pDB951. Briefly, primers were used at 5ng/µl, and dNTPs at a final concentration of 0.2mM in a total reaction volume of 100µl. 2.5 Units of Taq polymerase enzyme from Advanced Biotechnologies were used and 31 cycles of PCR performed. Cycles consisted of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute 30 seconds at 50°C, and an elongation step of 1 minute 30 seconds at 72°C. On cycle 1 denaturation was extended to 5 minutes and on the final cycle elongation was extended to 7 minutes.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4) and 400bp (IL4.Y124D) were obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions were then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 and EcoRV and XhoI for IL4.Y124D. The digests were incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs were analysed by gel electrophoresis and gave sizes of approximately 690bp (hinge-CH2-CH3 of IgG4) and 370bp (IL4.Y124D).

A vector was prepared into which to ligate the hinge-CH2-CH3 of IgG4 and IL4.Y124D PCR fragments by digesting pDB951 (IL4.Y124D in COSFcLink) with EcoRV and XbaI to remove most of the IL4.Y124D/IgG1 fusion molecule. The only part remaining is approximately 75bp at the 5' end of IL4, which is not present in the IL4.Y124D EcoRV/XhoI fragment produced by PCR amplification. 5µg of pDB951 DNA was digested in a total volume of 30µl using react 2 buffer (GibcoBRL). The resulting 5.8Kb DNA fragment was purified using the Geneclean TM procedure.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) were ligated together to form plasmid pDB952, which encodes the IL4.Y124D/IgG4 fusion protein. The ligation was carried out using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16° C overnight. The ligation reaction products were transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants were cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 recombinant DNA was verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 regions were verified by DNA sequencing. Table 6 describes the sequence of the coding region only of the IL4.Y124D/IgG4 fusion molecule, and Table 7 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 recombinant DNA was prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. Expression of the fusion protein

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25µg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. Biological Activity

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell

mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG4 chimera inhibited ^{3}H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 3 IL4.Y124D/IgG4 PE fusion protein

1. Construction of DNA coding for fusion protein

PCR is performed to amplify the IL4.Y124D coding region and introduce a silent nucleotide substitution at the 3' end which creates a XhoI site as described in Example 2.

A second PCR reaction is performed to amplify the hinge-CH2-CH3 fragment of the human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

The IgG4 PE variant was created using PCR mutagenesis on the synthetic human IgG4 heavy chain cDNA described in Table 5, and was then ligated into the pCD expression vector. It was this plasmid which was used as the substrate for the PCR reaction amplifying the hinge-CH2-CH3 fragment of IgG4 PE. The sequence of the IgG4 PE variant is described in Table 8. The residues of the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:

referring to Table 8:

residue 322 has been altered to "C" in the PE variant from "T" in the wild type;

residue 333 has been altered to "G" in the PE variant from "A" in the wild type; and

residues 343-344 have been altered to "GA" in the PE variant from "CT" in the wild type.

Oligonucleotide primers are used for amplification of the IgG4 PE variant hinge-CH2-CH3 region as described for the derivation of pDB952.

PCR products of approximately 700bp (hinge-CH2-CH3 of IgG4 PE mutant) and 400bp (IL4.Y124D) are obtained and purified using the Promega "Magic PCR cleanup" kit. The purified PCR reactions are then digested with the following enzymes to create "sticky ends": XhoI and XbaI for IgG4 PE and EcoRV and XhoI for IL4.Y124D. The digests are incubated at 37°C for 3 hours and then ethanol precipitated. The resulting DNAs are of sizes of approximately 690bp (hinge-CH2-CH3 of IgG4 PE) and 370bp (IL4.Y124D).

To obtain larger amounts of the IgG4 PE variant hinge-CH2-CH3 fragment and the IL4.Y124D fragment, the purified and digested PCR products are ligated into Bluescript KS^{+TM} which is prepared by digestion with either XhoI and XbaI for the hinge-CH2-CH3 of IgG4 PE fragment or EcoRV and XhoI for the IL4.Y124D fragment, followed by Geneclean^{TM}. A Bluescript KS^{+}/hinge-CH2-CH3 of IgG4 PE recombinant and a Bluescript KS^{+}/IL4.Y124D recombinant are thus generated. Large amounts of these DNAs are produced using the Promega "Magic Maxiprep" method. The IgG4 PE hinge-CH2-CH3 fragment is excised from the Bluescript recombinant using XhoI and XbaI. The resulting fragment of approximately 690bp is purified by Geneclean^{TM} to generate large amounts of the IgG4 PE hinge-CH2-CH3 XhoI/XbaI fragment. The IL4.Y124D fragment is excised from the Bluescript recombinant using EcoRV and XhoI and the resulting fragment of approximately 370bp is purified by Geneclean^{TM}.

A vector is prepared into which to ligate the hinge-CH2-CH3 of IgG4 PE and IL4.Y124D fragments by digesting pDB951 with EcoRV and XbaI as described for the derivation of pDB952.

The three fragments described (IL4.Y124D EcoRV/XhoI, hinge-CH2-CH3 of IgG4 PE variant XhoI/XbaI and the 5.8Kb fragment resulting from EcoRV/XbaI digestion of pDB951) are ligated together to form plasmid pDB953 using a DNA ligation kit from Amersham (product code RPN 1507), incubating the reactions at 16°C overnight. The ligation reaction products are transformed into Promega JM109 competent cells (high efficiency) and plated onto Luria Broth agar containing ampicillin at 50µg/ml. Transformants are cultured in Luria Broth (containing ampicillin at 50µg/ml) and DNA prepared using Promega "Magic Minipreps". Production of an IL4.Y124D/IgG4 PE variant recombinant DNA is verified by restriction digests, and the complete IL4.Y124D and hinge-CH2-CH3 IgG4 PE variant regions are verified by DNA sequencing. Table 9 describes the sequence of the coding region only of the IL4.Y124D/IgG4 PE fusion molecule, and Table 10 contains the amino acid sequence of the fusion protein. The IL4.Y124D/IgG4 PE recombinant DNA is prepared and purified using caesium chloride gradients and the DNA used to transiently transfect HeLa cells.

2. **Expression of the fusion protein**

HeLa cells were grown in MEMα medium (Gibco) with 10% foetal calf serum and 1% glutamine. For the assay, 1 x 10^6 HeLa cells were seeded in 15mls RPMI-1640 medium with 10% newborn calf serum, 1% glutamine ("seeding medium"), in a 75cm^2 flask, four days prior to transfection. On the day prior to transfection, a further 12.5mls seeding medium was added to each flask. On the day of transfection, the medium was changed to 15mls of "transfection medium" (MEM medium with Earle's salts containing 10% newborn calf serum and 1% non essential amino acids), at time zero. At time +3 hours, 25μg of the appropriate DNA in 0.125M $CaCl_2$, 1x HBS (HEPES buffered saline) was added to the cells. At time +7 hours, the cells were subjected to a glycerol shock (15%v/v) and then left to incubate overnight in 12.5mls seeding medium containing 5mM sodium butyrate. The next day the cells were washed with PBS (Dulbecco's phosphate buffered saline) and 12.5mls "harvest medium" (RPMI-1640 with 2% of a 7.5% stock sodium bicarbonate solution) was added. After a further 24 hour incubation, the supernatants were removed, centrifuged at 1000rpm for 5 minutes to remove cell debris and stored at either 4°C or -20°C.

3. **Biological Activity**

For assay of supernatant for IL4 antagonist activity: using the method described in Spits et al., J. Immunology 139, 1142 (1987), human peripheral blood lymphocytes were incubated for three days with phytohaemagluttinin, a T cell mitogen, to upregulate the IL4 receptor. The resultant blast cells were then stimulated for a further three days with IL4. Proliferation was measured by the incorporation of 3H thymidine.

The IL4.Y124D/IgG4 PE chimera inhibited ^3H thymidine incorporation by human peripheral blood T lymphocytes stimulated with 133pM IL4 in a dose dependent manner.

Example 4. Mammalian Expression vector containing DNA coding for IL4.Y124D/IgG4 PE.

1. **Construction of DNA**

The pCDN vector (Aiyar, N., Baker, E., Wu, H-L., Nambi, P., Edwards, R.M., Trill, J.J., Ellis, C., Bergsma, D. Molecular and Cellular Biochemistry 131:75-86, 1994) contains the CMV promoter, a polylinker cloning region, and the BGH polyadenylation

region. This vector also contains a bacterial neomycin phosphotransferase gene (NEO) inserted between the β-globin promoter and SV40 polyadenylation region for Geneticin™ selection, the DHFR selection cassette inserted between the β-globin promoter and BGH polydenylation region for methotrexate (MTX) amplification, an ampicillin resistance gene for growth in bacteria, and a SV40 origin of replication.

To insert the IL4.Y124D/IgG4 PE cDNA, the pCDN vector was prepared by digesting with NdeI and BstX1 as follows: 15μg of DNA was incubated with 30 units of BstX1 in react 2 (Gibco-BRL) at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated. The IL4.Y124D/IgG4 PE fragment was prepared from pDB953 (Example 3.1) by digesting with BstX1 and NdeI as follows: 15μg of DNA was incubated with 30 units of BstX1 in react 2 at 55°C for 1 hour, and ethanol precipitated. The resulting DNA was digested with NdeI in react 2 at 37°C for 1 hour, and ethanol precipitated.

The IL4.Y124D/IgG4 PE NdeI/BstX1 and pCDN NdeI/BstX1 fragments were ligated together to form the plasmid pCDN-IL4.Y124D/IgG4 PE. The ligation was achieved using 2 units of T4 DNA Ligase (Gibco BRL) with T4 DNA Ligase buffer. The reactions were incubated at 16°C overnight. The ligation reaction products were transformed into Gibco-BRL DH5a competent cells (subcloning efficiency) and plated onto Luria Broth agar containing 75 ug/ml ampicillin. Transformants were cultured in Luria Broth (containing ampicillin at 50 ug/ml) and DNA prepared by alkaline lysis. Production of a pCDN-IL4.Y124D/IgG4 PE DNA was confirmed by restriction digests. The complete sequence of the recombinant IL4.Y124D/IgG4 PE DNA was confirmed by sequencing. The pCDN-IL4.Y124D/IgG4 PE recombinant DNA was prepared and purified using Qiagen columns and the DNA was used to transiently infect COS cells and electroporated into CHO cells to create stable clones.

2. Expression of the Fusion Protein

a) Transient Expression in COS

COS-1 cells were grown in DMEM medium with 10% fetal bovine serum. For the transfection, cells were seeded at 2 X 10^5 cells into a 35mm tissue culture dish 24 hours prior. A solution containing 1μg of DNA in100ul of DMEM without serum is added to a solution containing 6μl of LIPOFECTAMINE Reagent (Gibco-BRL) in 100μl of DMEM without serum, gently swirled and incubated at room temperature for 45 minutes. The cells are washed once with serum free DMEM. 0.8ml of serum free DMEM is added to the DNA-LIPOFECTAMINE SOLUTION, mixed gently and the diluted solution is overlayed on the cells. The cells are incubated at 37°C for 5 hours, then 1ml of DMEM containing 20% fetal bovine serum is added. The cells are assayed 48-72 hours later to determine expression levels.

b) **Electroporation into CHO cells**

CHO cells, ACC-098 (a suspension cell line derived from CHO DG-44, Urlaub, G., Kas, E., Carothers, A.M. and Chasin, L.A. Cell, 33. 405-412, 1983) were grown in serum free growth medium WO 92/05246. 15µg of the pCDN-IL4.Y124D/IgG4 PE plasmid was digested using 30 units of Not1 at 37°C for 3 hours to linearize the plasmid, and precipitated with ethanol. The resulting DNA was resuspended in 50ul of 1 X TE (10mM Tris, pH 8.0, 1mM EDTA). The DNA was electroporated into 1 X 10^7 ACC-098 cells, using a Bio Rad Gene Pulser set at 380V and 25µFd. The cells were resupended into growth medium at 2.5 X 10^4 cells/ml, and 200µl of the cell suspension was plated into each well of a 96 well plate. 48 hours later the medium was switched to growth medium containing 400µg/ml G418 (Geneticin). Twenty one days post selection, conditioned medium from the colonies which arose were screened by Elisa assay. The highest expressing colonies were transferred to 24 well plates in order to be scaled up.

Table 1. DNA sequence of COSFcLink vector, 6367bp

SEQ ID No:1

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ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGG     360
GACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG     420
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTC     480
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Table 2. DNA sequence of encoded Y124D-IgG1 fusion molecule in COSFcLink vector, 6926bp

SEQ ID No:2

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GTACTAAGGTCAAATCTAAGTGGATCAAGGAACTCCACATAAAACCAGAGACACTGAAAC 4260
TTATAGAGGAGAAAGTGGGGAAAAGCCTCGAAGATATGGGCACAGGGGAAAAATTCCTGA 4320
ATAGAACAGCAATGGCTTGTGCTGTAAGATCGAGAATTGACAAATGGGACCTCATGAAAC 4380
TCCAAAGCTATCGGATCAATTCCTCCAAAAAAGCCTCCTCACTACTTCTGGAATAGCTCA 4440
GAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGCATGGGG 4500
CGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGGCGGAGTTAGGGGCGGGAC 4560
TATGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGCTGGGGAGCCTGG 4620
GGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATGCTTTGCATACTTCTGCCTGC 4680
TGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTCCACAGAATTAATTCCCG 4740
ATCCCGTCGACCTCGAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATT 4800
GTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG 4860
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGT 4920
CGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTT 4980
TGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGC 5040
TGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGG 5100
ATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG 5160
CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGAC 5220
GCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG 5280
GAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCT 5340
TTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGG 5400
TGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCT 5460
GCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCAC 5520
TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT 5580
TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTC 5640
TGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCA 5700
CCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGAT 5760

```
CTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC  5820
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATT  5880
AAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACC  5940
AATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTG  6000
CCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTG  6060
CTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGC  6120
CAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTA  6180
TTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTG  6240
TTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCT  6300
CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA  6360
GCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGG  6420
TTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGA  6480
CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTT  6540
GCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCA  6600
TTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTT  6660
CGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTT  6720
CTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGA  6780
AATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATT  6840
GTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGC  6900
GCACATTTCCCCGAAAAGTGCCACCT                                     6926
```

Table 3. DNA sequence of IL4.Y124D/IgG1 fusion molecule coding region, 1164bp

SEQ ID No:3

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC    60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC   120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC   180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC   240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC   300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG   360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA   420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGTTCGAGCGGTACCGAGCCCAAATCGGCC   480
GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTC   540
TTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA   600
TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGAC   660
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC   720
CGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG   780
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA   840
GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAG   900
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG   960
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC  1020
GACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG  1080
AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGC  1140
CTCTCCCTGTCTCCGGGTAAATGA                                       1164
```

Table 4. Sequence of encoded IL4.Y124D/IgG1 fusion protein, 387aa

SEQ ID No:4

```
   1  MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
  51  LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
```

```
101  KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
151  CSSGTEPKSA DKTHTCPPCP APELLGGPSV FLFPPKPKDT LMISRTPEVT
201  CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH
251  QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSRDELTK
301  NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL
351  TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK*
```

Table 5. DNA sequence of synthetic IgG4 cDNA, 1006bp

SEQ ID No:5

```
GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG      60
AGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG     120
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA     180
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC     240
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC     300
AAATATGGTCCCCCATGCCCATCATGCCCAGCACCTGAATTTCTGGGGGGACCATCAGTC     360
TTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG     420
TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT     480
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC     540
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG     600
TGCAAGGTCTCCAACAAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAA     660
GGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG     720
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAG     780
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC     840
GACGGATCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG     900
AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC     960
CTCTCCCTGTCTCTGGGTAAATGAGTGTAGTCTAGATCTACGTATG                  1006
```

Table 6. DNA sequence of IL4.Y124D/IgG4 fusion molecule coding region, 1149bp

SEQ ID No:6

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC      60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC     120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC     180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC     240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC     300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG     360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA     420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA     480
TGCCCATCATGCCCAGCACCTGAATTTCTGGGGGGACCATCAGTCTTCCTGTTCCCCCCA     540
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC     600
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT     660
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC     720
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC     780
AAAGGCCTCCCGTCATCGATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG     840
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG     900
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG     960
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGATCCTTCTTC    1020
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC    1080
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG    1140
GGTAAATGA                                                       1149
```

Table 7. Sequence of encoded IL4.Y124D/IgG4 fusion protein, 382aa

SEQ ID No:7

```
   1  MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
  51  LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
 101  KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
 151  CSSESKYGPP CPSCPAPEFL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 201  VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
 251  GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL
 301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS
 351  RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*
```

Table 8. DNA sequence of IgG4 PE variant, 984bp

SEQ ID No:8

```
GCTAGTACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG    60
AGCACgGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCG   120
TGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA   180
GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC   240
TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC   300
AAATATGGTCCCCCATGCCCAcCATGCCCAGCgCCTGAaTTtgaGGGGGGACCATCAGTC   360
TTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG   420
TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT   480
GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC   540
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG   600
TGCAAGGTCTCCAACAAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAAGCCAAA   660
GGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG   720
AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAG   780
TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCC   840
GACGGaTCCTTCTTCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG   900
AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC   960
CTCTCCCTGTCTCTGGGTAAATGA                                       984
```

Table 9. DNA sequence of IL4.Y124D/IgG4 PE fusion molecule coding region, 1149bp

SEQ ID No:9

```
ATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTCTTCCTGCTAGCATGTGCCGGCAAC    60
TTTGTCCACGGACACAAGTGCGATATCACCTTACAGGAGATCATCAAAACTTTGAACAGC   120
CTCACAGAGCAGAAGACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCC   180
AAGAACACAACTGAGAAGGAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGCAGTTCTAC   240
AGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCAC   300
AAGCAGCTGATCCGATTCCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGGGCTTG   360
AATTCCTGTCCTGTGAAGGAAGCCAACCAGAGTACGTTGGAAAACTTCTTGGAAAGGCTA   420
AAGACGATCATGAGAGAGAAAGACTCAAAGTGCTCGAGCGAGTCCAAATATGGTCCCCCA   480
TGCCCACCATGCCCAGCgCCTGAATTTGAGGGGGGACCATCAGTCTTCCTGTTCCCCCCA   540
AAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGAC   600
GTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT   660
AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTC   720
```

```
CTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAAC  780
AAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG  840
CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTG  900
ACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG  960
CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGaTCCTTCTTC 1020
CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGC 1080
TCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTG 1140
GGTAAATGA                                                     1149
```

Table 10. Sequence of encoded IL4.Y124D/IgG4 PE variant fusion protein, 382aa

SEQ ID No:10

```
   1  MGLTSQLLPP LFFLLACAGN FVHGHKCDIT LQEIIKTLNS LTEQKTLCTE
  51  LTVTDIFAAS KNTTEKETFC RAATVLRQFY SHHEKDTRCL GATAQQFHRH
 101  KQLIRFLKRL DRNLWGLAGL NSCPVKEANQ STLENFLERL KTIMREKDSK
 151  CSSESKYGPP CPPCPAPEFE GGPSVFLFPP KPKDTLMISR TPEVTCVVVD
 201  VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN
 251  GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL
 301  TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS
 351  RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK*
```

CLAIMS

1. A soluble protein having IL4 and/or IL13 antagonist or partial antagonist activity, comprising an IL4 mutant or variant fused to least one human immunoglobulin constant domain or fragment thereof.

2. A compound according to claim 1, wherein at least one amino acid, naturally occuring in wild type IL4 at any one of positions 120 to 128 inclusive, is replaced by a different natural amino acid.

3. A compound according to claim 2, wherein the tyrosine naturally occurring at position 124 is replaced by a different natural amino acid.

4. A compound according to claim 3, wherein the tyrosine naturally occurring at position 124 is replaced by aspartic acid.

5. A compound according to any one of the preceding claims, wherein the immunoglobulin is of the IgG subclass

6. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG.

7. A compound according to claim 5, wherein the constant domain(s) or fragment thereof is the whole or a substantial part of the constant region of the heavy chain of human IgG4.

8. A compound according to claim 1, having the amino acid sequence represented by SEQ ID No:4, SEQ ID No:7 or SEQ ID No:10.

9. A process for preparing a compound according to any one of the preceding claims, which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

10. A process according to claim 9, which comprises:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and

iv) recovering said compound.

11. A DNA polymer comprising a nucleotide sequence that encodes a compound according to any one of claims 1 to 8.

12. A DNA polymer according to claim 11, which comprises or consists of the sequence of SEQ ID No:3, SEQ ID No:6 or SEQ ID No:9.

13. A replicable expression vector comprising a DNA polymer according to claim 11.

14. A host cell transformed with a replicable expression vector according to claim 13.

15 A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

16. A method of treating conditions resulting from undesirable actions of IL4 and/or IL13 which comprises administering to the sufferer an effective amount of a compound according to claim 1.

17. A compound according to any one of claims 1 to 8, for use in therapy.

18. A compound according to any one of claims 1 to 8, for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.

19. Use of a compound according to any one of claims 1 to 8 in the manufacture of a medicament for use in the treatment of conditions resulting from undesirable actions of IL4 and/or IL13.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/03036

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 C07K14/54 C07K16/00 C07K19/00 A61K38/19
A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 464 533 (BEHRINGWERKE) 8 January 1992 cited in the application see claims; examples ---	1-7,10, 11,13-19
Y	WO,A,93 10235 (SEBALD) 27 May 1993 cited in the application see the whole document ---	1-7,10, 11,13-19
Y	EMBO JOURNAL, vol. 12, no. 7, July 1993 EYNSHAM, OXFORD GB, pages 2663-2670, S.M. ZURAWSKI ET AL 'Receptors for Interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction ' see the whole document --- -/--	1-7,10, 11,13-19

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 1995	03.01.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOLECULAR IMMUNOLOGY, vol. 30, no. 1, January 1993 pages 105-108, S. ANGAL ET AL 'A singgle amino acid sustitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody' cited in the application see the whole document	1-7,10, 11,13-19
Y	WO,A,88 07089 (MEDICAL RESEARCH COUNCIL) 22 September 1988 see the whole document & EP,A,0 307 434 (MEDICAL RESEARCH COUNCIL) cited in the application	1-7,10, 11,13-19
A	EP,A,0 367 166 (TAKEDA CHEMICAL INDUSTRIES. LTD.) 9 May 1990 see claims	1

INTERNATIONAL SEARCH REPORT

ternational application No.
PCT/EP 95/03036

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 16
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although this claim is directed to a method of treatment of the human/animal body (Rule 39.1(iv), the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/EP 95/03036

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-464533	08-01-92	AU-B-	655421	22-12-94
		AU-B-	7935791	02-01-92
		CA-A-	2045869	29-12-91
		JP-A-	5247094	24-09-93
WO-A-9310235	27-05-93	DE-A-	4137333	19-05-93
		AU-A-	2928292	15-06-93
		CA-A-	2123315	27-05-93
		CZ-A-	9401185	15-12-94
		EP-A-	0613499	07-09-94
		HU-A-	66826	30-01-95
		JP-T-	7501522	16-02-95
		NO-A-	941681	06-05-94
WO-A-8807089	22-09-88	AU-B-	600575	16-08-90
		AU-B-	1480388	10-10-88
		DE-D-	3883899	14-10-93
		DE-T-	3883899	31-03-94
		EP-A,B	0307434	22-03-89
		EP-A-	0351410	24-01-90
		WO-A-	8807054	22-09-88
		GB-A,B	2209757	24-05-89
		JP-T-	1502875	05-10-89
EP-A-367166	09-05-90	AU-B-	622724	16-04-92
		AU-B-	4391089	03-05-90
		JP-A-	2209898	21-08-90